INSOLUBLE DRUGS AND DEVELOPMENT OF THEIR AQUEOUS INJECTABLE DOSAGE FORM

Thesis

Submitted in fulfillment of requirement

for the degree of DOCTOR OF PHILOSOPH

(Pharmacy)

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UTTAR PRADESH, INDIA

2007

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CERTIFICATE

This is to certify that the work entitled "Enhancement of aqueous solubility of water insoluble drugs and development of their aqueous injectable dosage form" is a piece of research work done by Mr. Akhilesh Jain, under our guidance and supervision for the degree of Doctor of Philosophy of Bundelkhand University, Jhansi (U.P.) India. That the candidate has put in an attendance of more than 200 days with us.

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DECLARATION

I declare that the thesis entitled "Enhancement of aqueous solubility of water insoluble drugs and development of their aqueous injectable dosage form" is my own work conducted under the supervision of Prof. S.K. Jain (Supervisor), Department of Pharmaceutical Sciences of Dr. Hari Singh Gour Vishwavidyalaya, Sagar (M.P.) and co-supervision of Dr. S.K. Prajapati (Co-supervisor), Institute of Pharmacy of Bundelkhand University, Jhansi (U.P.). I have put in more than 200 days of attendance with the supervisor/co-supervisor at the Institute of Pharmacy, Bundelkhand University, Jhansi (U.P.) of this University.

I further declare that to the best of my knowledge the thesis does not contain any part of any work, which has been submitted for the award of any degree either in this University or in any other University/Deemed University without proper citation.

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■ Acknowledgement ▶

It was all dark in the beginning of this long arduous, obscure journey, in the pursuit of reaching my goal, but the dawn was brought in my life by the grace, benediction and blessings of "Almighty God". In the path of achieving this venture, I was shaped, guided and rejuvenated by valuable ideas, criticism and suggestions of many people who assisted me to reach this destination. Although words are not enough to express my sincere gratitude but still I shall try to comprehend as far as I can.

Vocabulary runs out, words wither, language falters and emotions go berserk, when it comes to thanking the one who has been a constant source of inspiration in the most critical part of the hour, perhaps some feelings are best expressed when left unsaid, words been superfluous, yet I make an effort to acknowledge my esteemed guide **Prof. S. K. Jain**, Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar (M.P.), whose excellent and constant guidance, supervision, intellect vision and research skills helped in intellect vision and research skills helped in steering the present work through to its completion. His critical assessment, affectionate words and ever available patience always kept me charged and motivated me to perform best even in the situations of ups and downs.

My vocabulary fails to extend my esteemed regards, vast indebtedness and warm gratitude to my co-guide **Dr. S.K. Prajapati**, Reader, Institute of Pharmacy, Bundelkhand University, Jhansi (U.P.) for all the roles of care selfless support, his time-to-time cooperation and suggestions help me in completing this project. He had shown unique capacity of tolerance with dedication in guiding me. His brotherly support and invaluable suggestions were always there with me.

I offer my sincere gratitude to Dr. Raghuveer Irchhaiya, Head, Institute of Pharmacy, Bundelkhand University, Jhansi (U.P.) for providing the required facilities to carry out the research work comfortably.

I gratefully acknowledge all my colleagues of institute for being very helpful to me. I wish to acknowledge all the non-teaching personality of department for their "helping hand".

I personally acknowledge SIGNA Laboratories, Kanpur (U.P.) for providing me Chlorzoxazone and Indomethacin as gift sample.

I would like to thank Institutional Animal Ethical Committee, Institute of Pharmacy, Bundelkhand University, Jhansi (U.P.), for permission of *in vivo* studies.

I am also grateful to Prof. Ranjit Singh, HNB Garhwal University and Prof. Shailendra Saraf, NIEC Lucknow, for their suggestions and help to complete my work.

I can not forget to acknowledge my students Alka, Bhupesh, Chhater, Vaibhav, Avinash and lot.. for their help during my study.

Sometimes it becomes really hard to pick up the right words for those whose love and care are fathomless. All words could not sum up the contribution of my Parents, in my ascent to this achievement. They gave much more than I ever asked. Their love and care for me is pure and divine. "To walk on your path is my biggest dream, to follow your footsteps, my greatest need... For you are a wonderful person, so complete and true that I wish I become at least a shade like you".

There is something above all the relations, it is the golden thread that ties two heart, its a bit of special beauty of "Sneh", which enlightens the life, when the sun dippeth low. My wife, whom I can trust utterly and who comfort and encourage me in the day of difficulty and sorrow, when the world leaves me alone, to fight own battles. The innocent smile and naughtiness of 'Navi-Avi' helped me to take things easy in troubled times.

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Akhilesh K. Jain



Certificate Declaration

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Introduction

t is widely recognized that up to 40% of active pharmaceutical ingredients currently being developed are hydrophobic. Hydrophobicity of drug can delay or completely block new drug development and can prevent the much-needed reformulation of some currently marketed drugs. Finding a new drug candidate is even more complicated and costly then improvement of solubility and comprehensive ADME properties of existing drug. The delivery of water insoluble or poorly soluble drugs by injections is a recalcitrant problem facing pharmaceutical scientists. A major problem in the formulation of injectable drugs is that it is necessary that the desired dose of a water insoluble drug must be contained in the specified volume (1or 2 ml) of aqueous vehicle of the injection Therefore the hydrophobic drug must be solubilized so that the desired dose will dissolve in 1or 2 ml aqueous vehicle.

Solubility enhancement has broad implication in parenteral formulation design. The choice of a method of solubilization is dependent upon how efficiently the drug can be solubilized and upon the biocompatibility of the vehicle.

Drug solubilization has been a subject of many scientific articles and textbooks; yet despite this attention and available literature, product development scientist still encounters significant difficulties in their solubility problem. Theories of solute solubilization are not easy to understand. Solubilization processes are amazing, complex and require a fair amount of expertise in physical chemistry to interpret and apply current theoretical models. In order to understand what method to utilize for increasing the solubility of a poorly water-soluble drug, it is necessary to understand what is solution and solubilization and why a compound is insoluble.

A **solution** is defined as a mixture of two or more components forming a homogenous molecular dispersion. A solution can be a solid dissolved in another solid, a liquid, or a gas, the same being true for liquids and gases.

In order to prepare a solution, a chemical substance must be soluble in a liquid. The **solubility** of a substance at a given temperature is defined as the concentration of the dissolved solute, which is in equilibrium with the solid solute. Intrinsic solubility is defined as the maximum concentration to which a solution can be prepared with a specific **solute** and **solvent**. In a **saturated solution** containing undissolved solid solute, the rate at which the molecules or ions leave the solid surface is equal to the rate at which the solvated molecules return to the solid (Figure 1.1). K_{SOL} is the rate constant at which the solid is solvated and K_{PPT} is the rate constant at which the solvated molecule is returned to the solid. The solubility of a substance is the ratio of these rate constants at equilibrium in a given solution.

$$K solubility = \frac{K sol}{K ppt}$$

$$K sol \longrightarrow H_2 \circ \longrightarrow H_2$$

Fig. 1.1: A saturated solution at equilibrium

An unsaturated solution is a solution containing the dissolved solute in a concentration less than a saturated solution. Most pharmaceutical solutions are considered unsaturated. It is important for the pharmacist to control environmental conditions, which impact these types of solutions, since it is possible to impact the activity of the product.

Solutions used in pharmacy consist of a wide range of solutes and solvents. The basis of solubility and solution theory is based on **ideal solutions**. In an ideal solution, it is assumed there is a complete absence of attractive or repulsive forces and therefore the solvent does not affect the solubility. The solubility in this situation depends on temperature, the melting point of the solid and the molar heat of fusion ($\triangle H_f$) where the heat of solution is equal to $\triangle H_f$. This means no heat is formed or absorbed when mixing the solution. Therefore, the solubility in an ideal solution can be described by:

$$logX_{2}^{i} = \frac{\Delta H_{f}}{2.303 \times R} \times \frac{T_{o} - T}{T \times T}$$

Where X_2^i is the ideal solubility (as a mole fraction), R is the gas constant and T is the temperature of the solution (in Kelvin). This equation

can be used to calculate molar heat of fusion (ΔH_f) by plotting the logarithm of solubility against the reciprocal of the absolute temperature. This results in a slope of $-\Delta H_f$ /2.303R.

In a nonideal solution, mixing of solute and solvent can release heat into the surroundings or absorb heat from the surroundings. Activity (a) of a solute is defined as the concentration of the solute multiplied by the activity coefficient (γ^w). The activity coefficient is proportional to the volume of solute and to the fraction of the total volume occupied by the solvent. When this is substituted into the ideal solution equation, the resulting equation is:

$$\log X_2 = \frac{\Delta H_f}{2.303 \times R} \times \frac{T_0 - T}{T_0} + \log Y^w$$

where T_0 is the melting point of the solute. As the activity approaches infinity the solution become more ideal. For example, as a solution become more dilute the activity increases and the solution becomes ideal.

An additional term used in solubility is the **solubility parameter**. The solubility parameter (L) is a measure of cohesive forces between like molecules and is described by the two following equations:

$$\log \gamma_2 = (L_1 - L_2) \times \frac{V_2 \times \varphi_1^2}{2.303RT}$$

and

$$L = \left(\frac{\Delta H_{V} - RT}{V_{1}}\right)^{\frac{1}{2}}$$

where ΔH_v is heat of vaporization of the solute, V_1 is the volume per mole of the solute as a liquid, T is temperature (in Kelvin), R is the gas constant, V_2 is the molar volume of solute, and Φ_i^2 is the volume fraction of solvent.

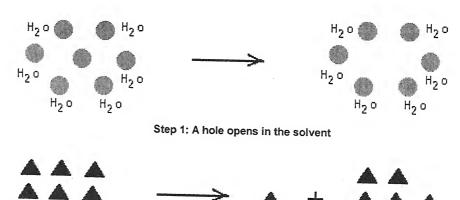
A **true solution** is defined as a mixture of two or more components that form a homogenous molecular dispersion.

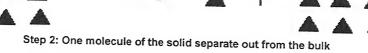
1.1 SOLUBILITY PRINCIPLES

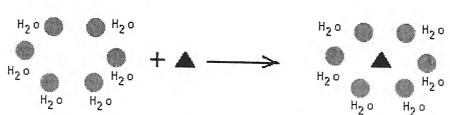
Atoms and molecules are held together by various types of bonds (e.g., London forces, hydrogen bonds, dipole-dipole, etc.). These forces are intricately involved in solubility because it is the solvent-solvent, solute-solute, and solvent-solute interactions that govern solubility.

1.1.1 Process of Dissolution

The process of **dissolution** involves the breaking of inter-ionic or intermolecular bonds in the solute, the separation of the molecules of the solvent to provide space in the solvent for the solute, and the interaction between the solvent and the solute molecule or ion.







Step 3: The freed solid molecule is integrated into the hole in the solvent

Fig. 1.2: Process of dissolution

1.1.2 Effect of Molecular Structure on Aqueous Solubility

The extent of deviation from ideality can be quantitated by the activity coefficient. The aqueous activity coefficient, γ^w , is related to the activity, a, and solubility by Eq (1):

$$w = a/X_w \qquad ...(1)$$

Where X_w is the mole fraction solubility in water. The activity of a component (at constant temperature T. Pressure P) is defined as the ratio of the effective concentration (fugacity or escaping tendency) of the solute in the solvent compared to its effective concentration in the pure liquid solute.

When two immiscible liquids are at equilibrium, the activity of each component is equal in both phases. Hence the activities of a pure organic solute, and its saturated solution in water, are equal, giving Eq.(2)

$$\gamma^{\mathsf{w}} \times \mathsf{X}_{\mathsf{w}} = \gamma^{\mathsf{o}} \times \mathsf{X}_{\mathsf{o}} \qquad \dots (2)$$

Where o represents the organic phase. If it is assumed that water is not appreciably soluble in the organic phase is assumed to be pure solute, the activity coefficient of the solute in itself, γ° , is also equal to unity. As a result, the right hand side of Eq.(2) can be approximated by unity, as in Eqs. 3 and 4.

$$X_{w} = 1/\gamma^{w} \qquad ...(3)$$

Or
$$\operatorname{Log} X_w = -\operatorname{log} \gamma^w$$
 ...(4)

Thus, the aqueous solubility of a liquid organic solute is inversely proportional to its aqueous activity coefficient.

Calculation of y

A common method for the determination of the aqueous activity coefficient utilizes the octanol/water partition coefficient of a drug, $K_{\text{o/w}}$. It is defined as the concentration of the drug in the octanol phase, C_{o} , over the concentration of the drug in the water phase, C_{w} , expressed by equation (5),

$$K_{o/w} = C_o/C_w \qquad ...(5)$$

Which can be related to the saturated solubility of the drug in each phase by Eqs. (6)

$$K_{o/w} = (X^{c}_{i} \times \gamma^{w}) / (X^{c}_{i} \times \gamma^{o}) \qquad ...(6)$$

Or
$$K_{o/w} = \gamma^w / \gamma^o$$
 ...(7)

For most nonpolar drugs it is appropriate to assume that is γ^{o} unity (i.e. octanol is an ideal solvent).

Hence, the activity coefficient in water γ^w , can be related directly to the octanol/water partition coefficient by Eq. (8)

$$K_{o/w} = \gamma^w$$
 ...(8)

Yalkowsky and Valvani¹ have shown that the molar activity coefficient can be given by Eq.(9)

$$\log \gamma^{W} = K_{o/W} + 0.80$$
 ...(9)

1.1.3 Effect of Crystal Lattice

A solute molecule must first dissociate from this crystal lattice before it can dissolve. Dissociation from the crystalline lattice is accompanied by a free-energy change. The more energy required to free a solute molecule from its crystal (i.e., the higher the melting point), the lower its solubility.

The dissolution of a solid solute can be considered as a two-step process. The first process is the breakdown of the crystal into a liquid at the temperature of interest. This liquid is referred to as a hypothetical supercooled liquid, which can be mixed with the solvent. In the case of an ideal solution, the two liquid phases mix freely without excess heat or volume and the solubility depends only upon the crystallinity of the solute.

Hence, in order to calculate the solubility of a solid solute, it is necessary to determine the free energy, ΔG , and necessary to produce a hypothetical supercooled liquid at a-given temperature². The molar ΔG can be obtained from an enthalpy-temperature thermodynamic cycle. Rigorously, it is necessary to sum the corresponding enthalpy and entropy changes that it takes to heat the crystal to the melting point, melt the crystal, and cool the liquid to the reference temperature. For the discussion here, it is convenient to omit any change in enthalpy with temperature (heat capacity); this allows for the simple mathematical expression in Eq. (10).

$$\log X_i^c = -\Delta S_m (T_m - T) / 2.303 RT$$
 ...(10)

Where X_i^c is the ideal solubility (mole fraction), R is the universal gas constant (1.98 cal/K-mol or 8.28 J/K-mol), ΔS_m , is the entropy of melting, and T_m is the melting point of the compound in Kelvin.

If the entropy of melting, ΔS_m , is not known, a reasonable approximation for rigid organic molecules of 13.5 cal/K-mol (56.5 J/K-mol) can be made³. If the temperature of interest is 298K, Eq. (10) changes to Eqs. (11) or (12),

$$\log X_i^c = -0.01(T_m - 298)$$
 ...(11)

or
$$\log X_i^c = -0.01(MP-25)$$
 ...(12)

Where MP is the melting point of the compound in Celsius.

It is important to emphasize that the ideal solubility is strictly a function of the pure crystal, and as a result, independent of the solvent. Equations (11) and (12) assume that the solvent does not effect the crystalline structure in any way to change the free energy. However, in aqueous systems, some drugs form hydrates, which generally lower the solubility in comparison to the anhydrous crystal. It is also interesting to note that hydrogen-bonding groups increase solubility with regard to activity coefficient (Table 1.1). However, hydrogen bonding also tends to raise the melting point, which, as shown above, decreases the solubility.

1.1.4 Effect of Molecular Structure and Melting Point

The first step in determining which technique to use for solubilizing a drug is to learn why the compound is poorly soluble. The above discussion shows, that there are two key components governing tile aqueous solubility of an organic solute in water: the molecular structure (activity coefficient) and the crystal structure (melting point). The aqueous solubility, X_w , of an organic nonelectrolyte is simply described by combining these two terms (Eqs. 4 and 10) to give Eq. (13).

$$\log X_{w} = \log X_{i}^{c} - \log \gamma^{w} \qquad ...(13)$$

Since the octanol/water partition coefficient if often known or estimated for new chemical entities, the solubility can be estimated by incorporating Eqs. (9) and (12) into Eq. (13) and converting to a molar basis, giving Eq. (I4),

$$\log S_{est} = -0.01(MP - 25) - \log K_{o/W} + 0.80$$
 ...(14)

Where S, is the estimated molar solubility, MP is the melting point (°C), and $K_{o/W}$ is the octanol/water partition coefficient. For drugs that are liquid at room temperature, the melting point term is zero, giving simply Eq. (15).

$$\log S_{est} = -\log K_{o/W} + 0.80$$
 ...(15)

It is important to remember that Eqs. (14) and (I5) are applicable to room temperature estimations of nonelectrolytes (note a negative-sign is in front of both the ideal solubility and $\log K_{o/W}$).

1.1.5 Energetics of Solubility

The actual solubility of a substance represents the total of the various factors involved in the transport of a solute particle from the solid phase to the solution phase. The driving force in dissolution is chiefly the interaction of the solvent molecules with solute molecules or the solute ions. For example, benzene is not soluble in water because the forces between the water molecules are so strong that they literally "squeeze out" the benzene. The net result of the interactions as the solute dissolves is manifested energetically as the *heat of solution* (ΔH_{SOL}). The end result is the equation:

$$Work = W_{22} + W_{11} - 2W_{12}$$

Where w_{22} is the work to separate solvent particles (Step 1 of Figure 1.2), w_{11} is the work to liberate a solute particle (Step 2 of Figure 1.2), and w_{12} is the interaction between the solute and solvent (Step 3 of Figure 1.2). The interaction term w_{12} is multiplied by 2 because it takes into the interaction of the solute-solvent and the work to close the hole in the solvent, the log w_{12} is a combination of work involved in solubilization, the volume of the solute and the volume occupied in the solvent. When the latter two factors are combined with the work of solubilization, we get the equation:

$$log\gamma_2 = (W_{22} + W_{11} - 2W_{12}) \times \frac{V_2 \times \Phi_1^2}{2.303RT}$$

Where V_2 is the molar volume of solute, and $\underline{\phi}_1^2$ is the volume fraction of solvent. For dilute solutions, $\underline{\phi}_1^2 = 1$. This means that dilute solutions can act as ideal solutions.

Step 1: Energy to separate solute particles

The first step in solubilization is the work required to separate solute particles. This is dependent on intermolecular forces of the solute. As mentioned the hydrogen bonds cause an attraction between molecules or intermolecular interactions. If –OH groups (or other hydrogen bonding moieties) of a molecule are in close proximity to each other, there can be intramolecular interactions. That is, a –OH on one side of the molecule will interact within the molecule to another hydrogen bonder. This intramolecular interaction would lower intermolecular interactions and lower melting temperature. Unfortunately, the addition of a polar group (e.g., -OH, NH₂) does not always increase solubility. Assume in the dissolution process that a solute molecule is removed from the solute phase without leaving a void. The energy associated with the removal of a single molecule is one-half the interaction energy between the two molecules of each interaction pair (i.e., ½ w₂₂ where w₂₂ is the interaction between 2 solutes).

Step 2: Energy to separate solvent molecules

In the next step of the dissolution process, a void large enough to receive a solute molecule is formed in the solution phase. This step is the work required to pull apart solvent molecules is described by ½ w₁₁ because this is equal to one-half the total interaction between a pair of solvent molecules. Again, intermolecular forces of the solvent are very important. As the structure of water (Figure 1.3), is highly ordered due to hydrogen bonding caused by the dipole nature of the molecule, water is a good solvent for polar molecules and has a high *dielectric constant*.

Fig. 1.3: Structure of water

The dielectric constant is a measure of the effect a substance has on the energy needed to separate two oppositely charged bodies. A vacuum is arbitrarily given a dielectric constant of 1. When we put two oppositely charged bodies into any medium, the medium tend to separate or make it more difficult for the two oppositely charged bodies to unite. The energy required to separate two oppositely charged bodies is inversely proportional to the dielectric constant of the medium.

Therefore, it requires only 1/81 (0.0123 times) as much energy to separate two charged bodies in water as in a vacuum, meaning it is easier to separate charge molecules in water than in a vacuum. The dielectric constant is also a measure of the degree of polarization in both an induced and permanent dipole. The dipole moment is a function of the charge and the distance between the charges. Associated molecules such as water and alcohol have high dipole moments and therefore high dielectric constants because of the long-chain pseudo-molecules.

Non-polar compounds like benzene do not have a sufficiently high dielectric constant to separate polar molecules like the ions of NaCl. These compounds can only dissolve those molecules held together by very weak intermolecular forces (induced dipole-induced dipole), such as naphthalene. Because there are very weak interactions (i.e., London forces) between solute-solute, solute-solvent, and solvent-solvent, these types of non-polar solutions behave near ideally.

Table 1.1: Dielectric Constants of Some Liquids at 20°C

Substance	Dielectric Constant
Carbon Tetrachloride	2.24
Benzene	2.28
Ethyl Ether	4.34
Chloroform	4.8
Ethyl Acetate	6.4
Phenol	9.7
Acetone	21.4
Ethanol	25.7
Methanol	33.7
Water	80.4
N-Methylformamide	190

Water, on the other hand, cannot dissolve things like naphthalene because the attraction of water for naphthalene is much less than that of water for water. The classification of solvents on the basis of polarity is often referred to as the rule of "like dissolves like." In other words, a highly polar or ionic compound dissolves in solvent that is also highly polar or has a high dielectric constant. And a compound that is non-polar dissolves in solvent that is relatively non-polar or has a low dielectric constant.

Step 3: Energetics between solvent and solute

Last, the solute molecule is placed in the void in the solution phase. The total interaction energy attributed to the interaction of a single solute molecule with the solvent is w_{12} . If the sizes of the solute and solvent molecules are similar, the net energy for the dissolution process may be expressed by the equation:

$$Work = W_{22} + W_{11} - 2W_{12}$$

If this process is to proceed, work should be a negative value.

Dipole-dipole interactions are responsible for the dissolution of many pharmaceutical agents. The solubility of the low molecular weight organic

acids, alcohols, amides, amines, esters, ketones and sugars in polar solvents is a result of dipole-dipole interactions. Since water is the one of the most commonly used solvents, hydrogen bond formation is often observed.

Alcohols dissolve in water because of hydrogen bonding. When the alkyl chain of a monohydric alcohol exceeds five carbons, the polarity is reduced and the longer alkyl chain alcohols are no longer water-soluble unless the hydrocarbon is branched. Alcohols (e.g., ethanol) are capable of dissolving polar solutes that are not soluble in water. Ethanol is often used as co-solvent with water to increase solubility of some hydrophobic molecules (e.g., elixirs).

As the number of hydroxyl groups (or other polar groups) increases on a molecule, there is often an increase in the water solubility. Polyhydric alcohols such as glycerin, mannitol, and sorbitol are polar and highly water-soluble. Phenols dissolve in water, glycerin and alcohol. As the ratio of hydroxy groups to the number of carbon atoms increases, the water solubility is increased. The solubility of resorcinol in water is about 15 times greater than phenol in water.

In strong dipole-dipole interactions, the solute-solvent interaction may exceed both the solvent-solvent interaction and solute-solute interaction, resulting in excess energy produced in the form of heat. The dissolution process is termed an exothermic reaction. Such a system has a negative heat of solution since the heat of solution is defined as the heat absorbed per mole dissolved.

Usually substances with a large negative heat of solution (i.e., exothermic reaction) are more soluble than substances with a smaller negative heat of solution. Compounds that have a positive heat of solution (endothermic) may also be soluble. With certain dipole-dipole interactions, the solute-solute and solvent-solvent interaction may exceed the energy provided from the solute-solvent interaction. To complete the dissolution process, thermal energy is absorbed from the environment. This dissolution process is known as endothermic and the heat of solution is positive.

Although the heat of solution is indicative of the solubility of a substance, other factors affect solubility. The spatial arrangement of a solute molecule in a crystal may decrease the movement from the solid phase into solution. As a general rule, crystals composed of unsymmetrical molecules are more soluble than those composed of highly symmetrical molecules. Therefore, amorphous solids are usually more soluble than crystalline counterparts.

1.1.6 Electrolytes Solubility

Dipole-ion interactions are the forces responsible for the dissolution of electrolytes in polar solvents. The attraction of an electrical center of a dipole to an oppositely charged ion often releases energy. The released energy is used to break the ion-ion bonds in the solid. Non-polar and weakly polar liquids do not have sufficiently strong interactions to dissolve electrolytes.

There are several traits that a compound should have in order to be a good electrolyte solvent. First, a compound should have a high dipole moment. Second, it should have a small molecular size to enable the dipole to approach closely to the electrolyte ions. It should also have a high dielectric constant, which reduces the intensity of electrical interaction between solvated ions. Water has all of these qualities.

1.1.7 Hydrates, Solvates, and Amorphous Compounds

In aqueous solutions, most ions are surrounded or hydrated by as many water molecules as the size of the ion allows. Anions are generally less highly hydrated than cations. The extent of interaction between ions and dipoles depends on the size and charge of the ions. It is interesting to note that the quantity of water in a crystal affects the heat of solution. In a crystal hydrate, the ions are largely hydrated, and consequently the ion-dipole interaction energy is considerably less than that of the anhydrous solute during the dissolution process. The hydrates, therefore, usually have lower water solubility. Table 1.2 gives apparent solubility ratios for some solvates. It can be seen that the transient solubility increase for solvates having solubility ratios ranging from 1 to 3. However, significant increases of nearly 20 and 30 times have been observed for calcium gluceptate and glibenclamide^{6, 17}.

Table 1.2: Apparent Solubility Ratios of Hydrates/Anhydrates and for Solvates/ Nonsolvates in Water between 25-37°C

Drug (Temp. °C)	Solvate/Nonsolvate Ratio	Ref.
Ampicillin (not cited)	anhydrate/trihydrate = 1.3	4
Ampicillin (20)	anhydrate/hydrate = 2.2	5.
Ampicillin (30)	anhydrate/hydrate = 1.5	5
Calcium gluceptate (37)	anhydrate/3.5 hydrate = 18.	6
Erythromycin (30)	anhydrate/dehydrate = 2.2	7
I.amivudine (25)	anhydrate/0.2 hydrate = 1.2	8
Paroxctine HCI (20)	anhydrate/hemi hydrate=1.7	9
Phenobarbital (20)	anhydrate/hydrate = 1.1	10
Phenobarbital (25)	anhydrate/hydrate = 1.4	10
Phenobarbital (35)	anhydrate/hydrate = 1.0	10
Piroxicam (25)	monohydrate/anhydrate = 1.0	11
Sulfamethoxazole (25)	anhydrate/monohydrate = 1.2	12
Theophylline (25)	anhydrate/monohydrate = 2.0	13
Uric acid (25)	hydrate/anhydrate = 1.6	14
Uric acid (37)	hydrate/anhydrate = 1.9	14
DMHP (2S) ^a	formate solvate/anhydrate = 8.2	15
Furosemide (37)	dioxane solvate/anhydrate = 1.3	16
Furosemide (37)	DMF ^b solvate/anhydrate = 1.2	16
Glibenclamide (37)	pentanol solvate/anhydrate = 31.8	17
Glibenclamide (37)	toluene solvate/anhydrate = 2.4	17

^aDMHP= 1,2-dimethyl-3-hydroxy-4-pyridone.

Table 1.3: Apparent Solubility Ratios of Amorphous Compounds

Solute	Solubility Ratio	Ref.
Caffeine	5	18
Heroin	16	18
Theophylline	50	18
Theobromine	50	18
Morphine	270	18
Hydrochlorthiazide	1,1	19
Bendrofluazide	2.8	19
Cyclothiazide	6.2	19
Cyclopenthiazide	8.3	19
Polvthiazidc	9.8	19

^bDMF = dimethylformamide.

Amorphous materials clearly have the highest free energy compared to any crystalline solid fraction. As a result, amorphous materials can be expected to give the highest apparent solubility enhancement. Toffoli et al. 18 and Corrigan et al. 19 have investigated amorphous materials and found a wide range of apparent solubility ratios. Some of their data listed in table 1.3 clearly show that the use of an amorphous material has the greatest potential for solubility enhancement.

1.1.8 Effect of Polymorphs

A solid has a rigid form and a definite shape. The shape or **habit** of a crystal (Fig. 1.4) of a given substance may vary but the angles between the faces are always constant. A crystal is made up of atoms, ions, or molecules in a regular geometric arrangement or lattice constantly repeated in three dimensions. This repeating pattern is known as the **unit cell**.

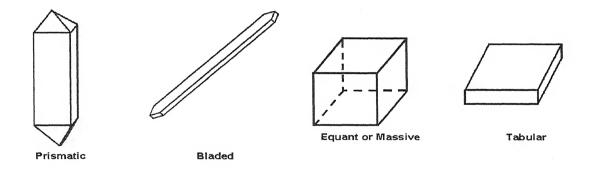


Fig. 1.4: Some examples of crystal habits

The capacity for a substance to crystallize in more than one crystalline form is **polymorphism**. It is possible that all crystals can crystallize in different forms or polymorphs. The color, hardness, solubility, melting point, and other properties of a compound depend on its polymorphic form. If the change from one polymorph to another is reversible, the process is called **enantiotropic**. If the system is **monotropic**, there is a transition point above the melting points of both polymorphs. The two polymorphs cannot be converted from one another without undergoing a phase transition.

Table 1.4: Solubility ratio of polymorphs

Compound	Solubility ratio	Ref.
Acemetacin	1.4	20
	2.0	20
	2.3	20
Acemetacin	2.0	21
	3.7	21
	2.6	21
	4.7	21
Acetazolamide	1.1	22
Acetohexamide	1.2	23
Aspirin	1.2	24
	1.3	24
Barbital	1.2	25
Benzoxoprofen	1.5	26
Cabamazepine	1.2	27
Chlortetracycline	1.5	28
Codeine	1.0	29
	5.1	29
Diisopropamide	1.0	30
Fusemide	1.6	31
Glibenclamide	1.6	32
Indomethacin	1.4	33
Mebendazole C/A	3.6	34
B/ A	7.4	34
Mefloquine HCI	1.2	35
Meprobamate	1.9	36
Nimodipine	2.0	37
Oxyclozamide II/I	2.6	38
111/1	3.9	38
Phenobarbital	1.1	39
Progesterone	1.2	40
Piretanide	1.6	41
Sulfanilamide	1.2	42
Sulfathiazole	1.8	43
Sulfono	1.1	43
Tetracycline	1.6	44
Tolbutamide	* 1.3	45
Tromexan	1.7	43

As mentioned, polymorphs can vary in melting point. Since the melting point of the solid is related to solubility, than polymorphs will most likely have different solubilities. If the wrong polymorph is chosen during the formulation process, the metastable (i.e., thermodynamically unstable form) form can convert to the stable form, which can result in changes in solubility. In order to assess the relative increase-in solubility of a polymorph with respect to

another, a simple **solubility ratio** can be defined. The solubility ratio is defined as the solubility of the metastable polymorph divided by the solubility of the more stable form. Table 1.4 gives a list of solubility ratios for different crystal forms of drugs.

1.1.9 Effect of Temperature

Often the solubility of a material increases by raising the temperature of the solvent, although there are some substances, which are more soluble in cold temperatures than in warm. When a substance dissolves it separates the intermolecular forces with surrounding molecules. Separation of molecules requires a certain amount of energy, which can be provided in the form of heat. There is also the possibility that the compound will form a bond with the solvent, resulting in energy release.

Non-polar Compounds

There is essentially no detectable heat effect in non-polar substances. The forces holding the particles together are small, and any interaction between solute and solvent is small.

Polar Substances

In polar substances, it takes energy to separate the molecule from surrounding molecules. This energy is supplied in the form of heat, producing a cooling effect. On the other hand, there is the possibility of interaction between the solute and solvent with formation of a dipole-dipole type bond, and this interaction will tend to give off heat. Depending on which of the two interactions is greatest an increase or decrease in temperature of the solution is observed.

1.2 SOLUBILIZATION APPROACHES

Various approaches to enhance water-solubilities are:

1.2.1 Solubilization by pH Control

1.2.1.1 Nonionizable substances

There is little effect of pH on nonionizable substances. Nonionizable, hydrophobic substances can have improved solubility by changing the dielectric constant of the solvent by the use of co-solvents rather than the pH of the solvent.

1.2.1.2 Ionizable substances

For substances that have an ionizable like a carboxylic acid (HA), solubility is a function of pH (Fig. 1.5). If the drug molecule is ionizable, then adjusting the pH of the system may be the simplest and most effective means of increasing aqueous solubility. Current FDA approved marketed parenteral range in pH from 2 to 11. Thus the molecule with pKa between 3-11 can be potentially solubilized by pH adjustment.

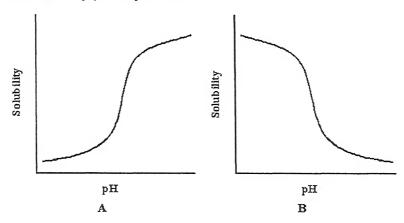


Fig. 1.5: The effect of pH on (A) a weak acid and (B) a weak base

1.2.1.3 Mathematical description

Equations (1) and (2) describe the behavior of a weak monoprotic acidic drug, HA_{(solid/liquid),} in water:

$$HA_{(solid/liquid)} \leftrightarrow HA_{(solution)}$$
 ...(1)

$$HA_{(solution)} + H_2O \leftarrow K_2 \rightarrow H_3O^+ + A^- \qquad ...(2)$$

Where HA_(solution) represents the free acid (unionized form) in solution and A⁻ the ionized acid in solution. The total concentration of drug in solution (S_T) is equal to [HA_(solution)] + [A⁻], where HA_(solution) is the intrinsic solubility of the drug (S_W). From Eq. (2) the ionized concentration of drug can be derived via Eq. (3),

$$[A^{-}] = \frac{K_a [HA]}{[H^{+}]}$$
(3)

$$[A^{-}] = \frac{K_a [HA]}{[H^{+}]}(3)$$

$$S_r = S_w + \frac{K_a [HA]}{[H^{+}]}(4)$$

Since [HA] = S_W , log K_a = pKa, and log H^+ = pH, Eq. (4) can be simplified to Eq. (5)

$$S_T - S_W (1 + 10^{(pH-pKa)})$$
(5)

Which relates the total solubility to the intrinsic solubility, the pKa of the weak monoprotic acid, and the pH of the system.

For a weak base in an aqueous solution, Eqs. (6) and (7) hold.

$$B_{\text{(solid/liquid)}} \leftrightarrow B_{\text{(solution)}} \dots (6)$$

$$B_{\text{(solution)}} + H2O \leftarrow K_b \rightarrow OH^- + HA^+ \qquad ...(7)$$

By analogy to the weak acid, the total solubility is described by Eq. (8),

$$S_T = S_W (1 + 10^{(pKa-pH)})$$
 ...(8)

Where pKa refers to HA⁺. Solubilization by ionization can be very efficient for a monoacidic or monobasic drug. The linear solubilization slope corresponds to a ten-fold increase in solubility for a change of one unit in pH. Zwitterion compounds, which have both acidic and basic functional groups, have a minimum solubility at a pH that is equal to the average pKa⁴⁶⁻⁴⁹. The solubilization of divalent acids and bases is similar to their monoprotic counterparts. However, upon the ionization of the second functional group, the solubilization slope is two instead of one, which corresponds to a 100 fold increase in solubility for a change of one unit in pH. A complete mathematical description for the solubilization of a dibasic compound is given by Ganen and Pyter ⁵⁰.

1.2.1.4 Salt formation

Usually, the first approach used to increase the water solubility of an insoluble drug in water is to form more water-soluble salts. Berge & Co-worker⁵¹ wrote what is now a near classic review of salt form strategies acceptable for pharmaceuticals. Equations (5) and (8) assume that the ionized species of a solute has infinite solubility. However, an ionized solute can form salts with appropriate counterions. Kramer and Flynn⁵² demonstrated how Eq. (8) can be modified to take salt formation into account. The formation of a salt is governed by the solubility product, K_{sp} , of the salt complex. For example, the thermodynamic equilibrium for a chloride salt, BH^+Cl^- , is given by Eq. (9)

$$BH^{+}CI^{-}_{(Solid)} \leftarrow K_{SP} \rightarrow [BH^{+}] + [CI^{-}] \qquad ...(9)$$

Where BH⁺Cl⁻(solid) represents the solid chloride salt and BH⁺ the ionized base, and Cl⁻ is the chloride counterion. As a result, the concentration of the ionized species is limited by the solubility of the salt. Some organic salts are very soluble in aqueous systems; others are not and can significantly limit the solubility of a solute.

In addition to overall solubility, salts can have a significant impact on the dissolution rate of a solute. Hence, solubility considerations are important factors to be assessed when choosing the appropriate pharmaceutical salt^{51, 53-57}.

1.2.1.5 Buffers

The practical use of a buffer is to simply maintain the pH of the system over time. For drugs solubilized by changing the pH, another practical use of a buffer is to reduce or eliminate the potential for precipitation of the drug upon dilution. Drug precipitation upon intravenous injection has been linked to phlebitis⁵⁸⁻⁶². As shown by the previous discussion, the solubility of a drug can be increased exponentially by changing the pH. However, it follows that the solubility can also decrease exponentially. If a pH-adjusted formulation is diluted with a medium by one half, the drug concentration is reduced by half. At the same time, the pH of the new mixture may change. If it changes by one pH unit in a direction that reduces the ionization of the drug, the solubility of that drug decreases by ten-fold. The drug can precipitate if the concentration in the solution exceeds the new solubility

1.2.1.6 Practical solubilization by pH control

Under the proper conditions, the solubility of an ionizable drug can increase exponentially by adjusting the pH of the solution. From a structural point of view, a drug that can be efficiently solubilized by pH control should be either a weak acid with a low pKa or a weak base with a high pKa. The effect of pH on solubilization is independent of the solubility of the unionized form of the drug.

Although the use of a buffer aids in reducing the risk of precipitation upon dilution, there are limitations to pH, concentration, and buffer type. Physiological compatibility depends on the route of administration, tonicity, and contact time. Other factors such as chemical stability as a function of pH must also be considered. Table 1.5 lists commercial parenteral products and associated buffers, concentrations, and pH values.

1.2.2 Solubilization by the Use of Cosolvents

If the drug molecule is nonionizable pH adjustment is insufficient in achieving the desired solution concentration, then the addition of a water-miscible or partially miscible organic solvent (i.e., cosolvent) to water is a common and effective way to increase the solubility. The chosen solvent must be haemocompatible, miscible with water—and body fluids, non-toxic, non-irritant, non-sensitizing, must not exert any pharmacological activity and viscosity should allow for easy injection.

Cosolvents are employed in approximately 10% of FDA approved parenteral products. They are useful because they may often provide exponential increase in solubility⁶⁴ and allow exclusion of water for compounds susceptible for hydrolysis. In recent years, surveys of FDA approved parenteral products⁶⁵⁻⁶⁷. Shows five water miscible cosolventspolyethylene glycol glycerin, ethanol, propylene glycol, and N. N- dimethylacetamide as component of sterile formulation. Toxicity and adverse clinical effects of common cosolvents are frequently reported and summarized by Smolinsky⁶⁸.

In order for an organic compound to be appreciably miscible with water, it must have some degree of hydrogen bond donating and/or hydrogen bond accepting ability. In addition, these organic structures also have small hydrocarbon regions. As discussed earlier, water interacts very strongly with other water molecules. Cosolvents with hydrocarbon regions do not interact as strongly. Thus, the introduction of a cosolvent to water decreases the water-water interactions and the ability of water to "squeeze out" a non-polar organic solute in the cosolvent system.

Table 1.5: Commercial parenteral product containing buffers^a

Trade-Name	Active Drug component	Manufacturer ^b	Route of Adminis. ^c	Buffer and Conc. (% w/v)	Нф
Thorazine	Chloropromazine HCI	SKF	IM/IV Infusion	Acetic acid, 0.2%	
Sus-Phrine	Epinepherine	Berlex	SC	Acetic acid, 1.0%	
Serpasil	Reserpine	Ciba	IV/IM	Adipic acid, 1.0%	3.0-4.0
Serpasil	Reserpine	Ciba .	IV/IM	Ascorbic acid, 0.05%	3.0-4.0
Sus-Phrine	Epinepherine	Berlex	SC	Ascorbic acid, 1.0%	
Duranest	Etidocaine HCI	Astra	Infiltration	Citric acid, 0.02%	3.0-4.5
VePesid	Etoposide	Bristol	IV Infusion	Citric acid, 0.2%	3.0-4.0
Vasoxvi	Methoxamine HCI	B-W	IV/IM	Citric acid (anhydrous), 0.3%	3.0-5.0
A+Idome ester HCI	Methyldopate HC1	MSD	IV Infusion	Citric acid (anhydrous), 0.5%	3.5-4.2
Nibain	Nalbuphine HCI	DuPont	IV/IM/SC	Citric acid, 1.26%	
Trilaton	Perphenazine	Schering	IV/IM	Disodium citrate, 2.46%	4.2-5.6
Taractan	Chlorprothixene HCI	Roche	M	Hydrochloric acid	3.4
Eractrate maleste	Fronovine maleate	Lilly	IV/IM	Lactic acid, 0.1%	2.7-3.5
Carlo in a carlo in a carlo		Sandoz	IV Infusion	Sodium acetate, 0.1%	3.7-4.3
Symposition		Roche	IM/IV	Sodium benzoate/benzoic acid5.0%	6.4-6.9
Valium	Diazepani) III	VI/VI/MI	Sodium bicarbonate, 3.0°/0	6.0-8.5
Ketlin	Cepnaloum southin	. Cib.	UV/IIM/SC	Sodium citrate, 0.65%	3.0-4.0
Priscoline	Tolazoline HCI -	Cina			0000
Adrucit	Fluorouracil	Adria	2	Sodium hydroxide	8.0-9.0
Oilantin	Phenytoin sodium	P-D	IV/IM	Sodium hydroxide	12.0
Cliantin	Trimethonrim Sulfamethoxazole	Roche	IV Infusion	Sodium hydroxide	10.0
bactriii	otoda milka o a in a company	Scharing	N//IM	Sodium phosphate, 1.0%	8.5
Celestone Phosphate	Betamethasone Souluin prospirate	B S S S S S S S S S S S S S S S S S S S		, , , , , , , , , , , , , , , , , , ,	3 0-4 0
Priscoline	Tolazoline HCI	Ciba	SC/IM/IV	lantanc acid, 0.03%	2:1

^bSKF = Smith-Kline-French; B-W = Burroughs-Wellcome; MSD = Merck Sharp & Dohme: P-D = Parke-Davis. ^aData from Ref. 63.

 c IV = intravenous; 1M = intramuscular; SC = subcutaneous.

From a thermodynamic standpoint, colsolvency predominantly affects the enthalpy of mixing. In other words, cosolvent are effective for compounds with poor aqueous solubilities due to large aqueous activity coefficients or, as related in Eq. (14, Section 1.1.4), for organic compounds that have large octanol/water partition coefficients. Once again, for nonpolar compounds, this is due to the poor molecular interactions between water and hydrocarbon regions. The appropriate choice of a cosolvent can result in a solvent system with physical properties that are more similar to those of the solute. Table 1.6 gives physical properties of commonly used pharmaceutical cosolvents in water. In the table n-octanol is added as a reference, since compounds with large octanol/water partition coefficients have poor aqueous solubility (i.e., interact more favorably in octanol than in water). Cosolvent systems with physical properties that are more similar to those of n-octanol would be expected to have greater success in solubilizing a non-polar drug. The data given in table 1.6 show that log K_{o/w} increases as

Table 1.6: Physical Properties of Common Cosolvents, Water, and n-Octanol

nonpolarity increases, whereas the other properties converge to a single

value as the nonpolarity increases. All of the parameters intercorrelate with

subtle differences for aprotic solvents. Aqueous solubilization via cosolvency

is dependent on both the solute and cosolvent physical properties.

Solvent	log K _{o/w}	Solubility Parameter	Surface Tension	Dielectric Constant
Water	-4.00	23.4	72.0	81.0
Glycerol	-2.60	16.5	64.9	42.5
Propylene glycol	-1.40	12.6	37.1	32.0
PEG-400	-1.30	11.3	46.0	13.6
Dimethyl sulfoxide	-1.09	12.0	38.0	46.7
Dimethylacetamide	-0.66	10.8	35.7	37.8
Ethanol	-0.31	12.7	22.2	24.3
n-Octanol	2.94	10.3	20.5	10.3

1.2.2.1 Mathematical description of cosolvency

It is difficult to predict or describe the effect that a particular cosolvent will have on a drug in an aqueous system. Several different methods have been proposed⁶⁹⁻⁸¹. Yalkowsky and co-workers ⁸²⁻⁸⁵ developed a practical cosolvent model. Yalkowsky and Roseman⁸³ derived Eq. (1) based on the assumption that the free energy of mixing a drug with a mixed solvent, ΔG_{mix} , is a linear combination of its free energy of mixing with the pure components (i.e., pure water, ΔG_{w} , and pure solvent, ΔG_{c}),

$$\Delta G \text{ mix} = f w \Delta G w + f c \Delta G c$$
 ...(1)

Where f_w and f_c are the volume fractions of water and cosolvent in the mixture, respectively. Converting to solubilities and assuming that the sum of the volume fractions of the two solvents is unity (i.e., the volume of the solute is negligible) gives Eqs. (2) or (3),

$$\log S_{mix} = (1 - f_c) \log S_w + f_c \log S_c \qquad \dots (2)$$

$$\log S_{mix} = \log S_w + f_c (\log S_c - \log S_w) \qquad ...(3)$$

Where S is molar solubility of the solute. Once again the solubility in water can be described by Eq.(4),

$$\log S_w = -0.01(MP - 25) - \log K_{o/w} + 0.8$$
 ...(4)

and by analogy the solubility of the solute in pure cosolvent by Eq. (5)

$$\log S_c = -0.01(MP - 25) - \log K_{o/c} + const.$$
 ...(5)

Where $K_{o/c}$ is the octanol cosolvent partition coefficient and the constant depends upon the molarity of the pure cosolvent. Inserting Eqs. (4) and (5) into the last term of Eq. (3) and simplifying, gives Eq. (6),

$$\log S_{mix} = \log S_w + f_c (\log K_{o/c} - \log K_{o/w} + 0.8 - \text{const.})...(6)$$

Which is not dependent upon the crystal properties of the solute since the melting point terms of Eqs. (4) and (5) cancel out. Thus, it is assumed that there are no solvent effects on the crystal, such as solvation or solvent-mediated phase transitions. In addition, $\log K_{o/c}$ and $\log K_{o/w}$ could be replaced with activity coefficients, although partition coefficients provide the most convenient method for the characterization of solutes.

Since all the terms in the bracket of Eq. (6) are constant for a given solute and cosolvent, they can be combined into a single constant, σ , giving Eq. (7).

$$\log S_{mix} = \log S_w + \sigma f_c \qquad ...(7)$$

Hence, as described by Eq. (7), the logarithm of the solubility of a nonpolar solute changes with cosolvent composition (f), having a slope of σ and an intercept of log S_w . Thus, on a linear scale, an exponential increase in solubility is observed with an increase in cosolvent composition. The key to solubilization by cosolvent is to determine which cosolvent gives the necessary σ . In order to optimize solubilization it is necessary to understand the parameters affecting cosolvent solubilization as shown below, σ is dependent upon the polarity of the drug and the polarity of the cosolvent.

From Eq. (7) it was shown that the slope of the solubilization curve could be related to the difference between the solute's octanol/water and octanol/cosolvent partition coefficients, as expressed in Eq. (8).

$$\sigma = \log K_{o/c} - \log K_{o/w} + 0.8 - \text{const.}$$
 ...(8)

Hansch and Leo⁸⁵ have shown that the partition coefficient of a series of solutes in one system can be correlated directly those of another system. Using this type of relationship for an octanol/water and octanol/cosolvent would give Eq. (9),

$$\log K_{o/c} = s \log K_{o/w} + t \qquad ...(9)$$

Where s and t are constants related to the polarity of the cosolvent. Inserting this relationship into Eq. (8) establishes a relationship, Eq. (9), between the solubilization slope and $\log K_{o/w}$

$$\underline{\sigma} = (S) \log K_{o/w} + T \qquad \dots (10)$$

Where T is the sum of all of the constants. Therefore the slope of the solubilization line is directly related to the polarity of the drug (via log $K_{o/w}$) and the polarity of the cosolvent (via S and T). For solid solutes, the melting point contributes only to the intercept of the solubilization profile. Since the intrinsic solubility does not directly affect the solubilization slope, it is convenient to rearrange Eq. (7) to Eq. (11).

$$\log \left[\frac{S_{\text{MIX}}}{S_{\text{w}}} \right] = \sigma f_{c} \qquad \dots (11)$$

By plotting $\log (S_{mix}/S_w)$ vs. f_c , solubilization lines are normalized with respect to the intrinsic solubility and pass through the origin on semi log plot.

1.2.2.2 Multiple cosolvents

Degree of solubilization attained for any solute is directly proportional to the octanol/ water partition coefficient of that solute. However, as with pH solubilization, solubilization through cosolvents is logarithmic, and as a result there is the potential for precipitation upon dilution.

A potential limitation to the use of cosolvents may be tile choice and amount of cosolvent needed. For pharmaceuticals, relatively few organic cosolvents are regarded as safe. Rubino⁸⁶ has discussed the biological effects of many of the commonly utilized cosolvents, such as ethanol, propylene glycol, glycerol, polyethylene glycols, and dimethylacetamide. The permissible amount of a given cosolvent depends upon the dosage form. Table 1.7 gives examples of products containing cosolvents.

1.2.3 Hydrotropes

Aqueous solubilization can also be achieved by addition of hydrotropic agents. The term hydrotropy refers to an increase in water solubility caused by addition of second compound, especially in large concentration.

The technique of hydrotropic solubilization is known since Neuberg reported in 1916 that increased aqueous solubility of organic substances normally insoluble of slightly in water, by addition of fairly high concentration of some alkali meta salts of various organic acids, which as for as known are non micelle forming compounds⁸⁷. Saleh et al.⁸⁸ extended definition of hydrotropic agents to include cationic and non ionic organic compounds bearing the essential structural features of Neuberg's hydrotropes.

Table 1.7: Parenteral Products Containing Cosolvents

Name	Component	Manufacturer	Adminstration ^a	% (w/v) Cosolvent
Serbasil	Reserpine	Ciba	IV/IM	10% Dimethylacetamide, 5% Polyethylene glycol
Tridil	Nitroglycerin	Amer. Critical Care	IV Infusion	30% Ethanol
VePesid	Etoposide	Bristol	IV Infusion	30% Ethanol, 13% Polyethylene glycol 300
Nembutal Sodium	Pentobarbital sodium	Abbot	IV/IM,	10% Ethanol, 40% Propylene glycol
Lanoxin	Digoxin	B-W	N/NI	10% Ethanol, 40% Prop ylene glycol
Berocca	Vitamins	Roche	IV Infusion	10% Ethanol, 40% Propylene glycol
Valium	Diazapam	Roche	IM/IV	10% Ethanol, 40% Propylene glycol
Dilantin	Phenytoin sodium	P-D	IMIIV	10% Ethanol, 40% Propylene glycol
Cedilanid-D	Desalanoside	Sandoz	/IM/IV	9.8% w/w Ethanol15% Glycerol
Pantopon	Opium alkaloids HCL	Roche	IM/SC	6% Ethanol, 13% Glycerol
Sus-Phrine	Epinephrine	Berlex	SC	32% Glycerol
Robaxin	Methocarbamol	Robins	IV/IM/IM Inf.	50% Polyethylene glycol 300
Actavín	Lorazepam	Wyeth	MI/AI	18% Polyethylene glycol 400
Apresoline HCI	Hydralazine HCL	Ciba	IM/IV	10% Propylene glycol
M.V.L-12	Multivitamin	Armour	IV Infusion.	30% Propylene glycol
Berocca	Vitamins	Roche	IV Infusion	40% Propylene glycol
Dramamine	Dimenhydinate	Searle	IM/IV	50% Propylene glycol
Terramycin	Oxytetracycline	Pfipharmecs	M	67-75% Propylene glycol
Loxitane	Loxapine HCL	Lederle	M	70% Propylene glycol

^aIV = intravenous, IM = intramuscular, SC = subcutaneous.

The mechanism by which this effect occurs is not clear. Some workers have speculated that hydrotropy is simply another type of solubilization with solute dissolved in oriented clusters of the hydrotropic agents. However, hydrotropic solutions do not show colloidal properties. Others feel that the most commonly proposed mechanism is complexation, but complexation does not explain all hydrotropic systems. A characteristic that many hydrotropic agents share is the ability to self-associate in a solution, particularly at hydrotropic concentration. The correlations between hydrotropicity and self-association and the mechanistic implication have not been exposed. Still other reason, that the phenomenon must be due to a change in solvent character due to large amount of additive needed to bring about the increase in solubility^{89,90,91}.

Table 1.8: List of Drugs studied for Hydrotropic Solubilization

S. No.	Drugs	Hydrotropic agents	
1.	Sulphapyridine, Sulfathiazole	Caffeine	92
2.	Hydrocortisone, Phenacetin, Prednisolone, Theophylline	Benzoic acid, m-hydroxy benzoic acid, p-hydroxy benzoic acid, Sodium benzoate, Sodium salicylate, Sodium methyl hydroxy benzoate, Sodium p- hydroxy benzoate	93
3.	Riboflavin	Ascorbic acid, Sodium ascorbate	94
4.	Barbiturates, Codeine derivatives, Purine bases	Sodium benzoate, Sodium p-toluene sulphonate, Sodium salicylate,	95
5.	Tropane derivatives	Sodium benzoate, Sodium m-amino benzoate, Sodium o-amino benzoate, Sodium m-hydroxy benzoate, Sodium o-hydroxy benzoate, Sodium p-hydroxy benzoate, Sodium p-methyl benzoate	96
6.	a) Adrenochrome mono semicarbazide b) Calcium carbonate c) Oxytetracycline dihydrate d) Theobromine e) Theophylline	Calcium glucoheptonate, Calcium lactobionate, Calcium levulonate Sodium p-amino benzoate, Sodium saccharine, Sodium salicylate Sodium acetate, Sodium salicylate Adrenosine, Piperazine, sodium salicylate	97
7.	Acetohexamide	Sodium benzoate, Sodium salicylate	98
8.	Menadione	Sodium benzoate, Sodium salicylate	99
9.	Chartreusin	Sodium m & p-hydroxy benzoate, Sodium benzoate, Sodium 2, 4/2, 5/2, 6/2, 4, 6, hydroxy benzoate	100
10.	Amidopyrine	Salicylic acid, Sodium benzoate, Sodium salicylate	101

S. No.	Drugs	Hydrotropic agents	
11.	Diazepam	Sodium salicylate	102
12.	Hydrocarbons	P-toluene sulfonic acid, Urea, methyl urea, 1,3-di methyl urea, Tetra methyl urea	103
13.	Riboflavin	Sodium-o-toluate, Sodium-p-toluate	104
14.	a) Hydro chlorthiazide b) Chlorthiazide	Sodium m & p-hydroxy benzoate, sodium salicylate Gentesic acid ethanolamide, Salicylic acid ethanolamide, Sodium anthranilate, Sodium pamino benzoate, Sodium gentisate, Sodium pamino gentisate, Sodium pyrocatechuate,	105
15.	Bendro quinones	Sodium p-amino salicylate Sodium benzoate, Sodium p-hydroxy benzoate, Sodium salicylate, Sodium p-amino salicylate	106
16.	Clonazepam, Diazepam, Medazepam, Nitrazepam, Oxazepam	Sodium salicylate	107
17.	Paracetamol	Sodium gentisate, Sodium glycinate, Sodium salicylate, Nicotinamide	108
18.	Esters of glycolic acid, Malonic acid, Lactic, Maleic, Aspartic & Glutamic acid	Urea	109
19.	Substituted benzoic acid	Sodium benzoate, Sodium salicylate	110
20.	Nifedipine	Sodium benzoate, Sodium salicylate	111, 112
21.	Ibuprofen	Sodium benzoate, Sodium salicylate	
22.	Etoposide	Sodium benzoate, Sodium 2, 4/2, 5/2, 4, 6 hydroxy benzoate, Sodium salicylate	114
23.	Carbamazepine	Sodium benzoate, Sodium salicylate	115
24.	Nalidixic acid	Resorcinol, Sodium benzoate, Sodium salicylate	116
25.	Caffeine	Benzoic acid	117, 118
26.	Caffeine	Sodium benzoate, Sodium salicylate	119

1.2.4 Solubilization by the Use of Surfactants

Surface active agents are usually incorporated into parenterals to provide one of several desirable properties (i) increase drug solubilization through micellization (ii) to prevent drug precipitation upon dilution 120 (iii) improve the stability of drug in solution by incorporation of drug into micellar structure 121. (iv) in protein formulations, prevent aggregation due to liquid-air or liquid-solid interfacial interaction.

Detailed reviews of micelle structure characterization techniques and pharmaceuticals application have been published ¹²²⁻¹²⁴. Adverse reactions of tween 80 are reported by Eschalier et al. ¹²⁵. Toxicity of the surfactant reported in the literature prior to 1983 are summarized by Attwood and Florence ¹²³. Children and Newborns may be particularly sensitive to these agents and administration to this population is discussed by Danish and Kottke ¹²⁶.

Surfactants are compounds that have molecular structures with two distinct regions, a polar region and a nonpolar region. Solubilization by surfactants has been defined by McBain as the spontaneous passage of poorly water-soluble solute molecules into an aqueous solution of a surfactant, in which a thermodynamically stable solution is formed 127. The mechanism for this phenomenon has been studied quite extensively and involves the property of surface-active agents to form colloidal aggregates known as micelles. When surfactants are added to a liquid at low concentrations, they tend to orient at the air-liquid interface. As additional surfactant is added, the interface becomes fully occupied, and the excess molecules are forced into the bulk of the liquid. At still higher concentrations, the molecules of surfactant in the bulk of the liquid begin to form oriented aggregates or micelles (Figure 1.6); this change in orientation occurs rather abruptly, and the concentration of surfactant at which it occurs is known as the critical micelle concentration (CMC). Solubilization is thought to occur by virtue of the solute dissolving in or being adsorbed onto the micelle. A nonpolar drug, which is squeezed from the water, can locate within the micelle core. A semipolar drug can locate between or partially within the core and the mantle. Since the micelles are soluble in water, any drug incorporated into the micelle is also soluble in water. Surfactants has been extensively studied for their properties, and uses and discussed in a number of texts and chapters 128-131.

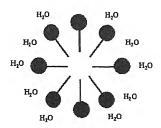


Fig. 1.6: Orientation of surface-active agent to form a spherical micelle

The ability of surfactant solutions to dissolve or solubilize water-insoluble materials starts at the critical micelle concentration and increases with the concentration of the micelles. The general solubilization curve for surfactants is given in Fig. 1.7. If the monomers of surfactant in solution do not affect the solubility of the solute, the solute concentration remains constant (at the intrinsic solubility, Sw) until the CMC is reached. Then the solute concentration increases linearly with increasing surfactant (micelle) concentration. A simple mathematical representation for the total solubility, S_T , of a solute in a surfactant system is given in Eq. (1),

$$S_T = S_W + K (C_{surf} - CMC)$$
 ...(1)

where C_{surf} is the total concentration of the surfactant and K the solubilization capacity. The quantity in parenthesis represents the micelle concentration. The solubilization capacity reflects the number of surfactant molecules required to solubilize a solutemolecule. Deviation to Eq. (1) is usually the result of micelle size or shape 'changes as the concentration of surfactant increases.

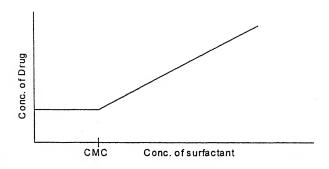


Fig. 1.7: General curve for solubilization with surfactants

1.2.4.1 Practical solubilization by surfactants

Although numerous anionic, cationic, and nonionic surfactants are available as solubilizing agents, only polysorbate-80 and cremophor EL have been used to any significant extent in parenteral products (Table 8). It should be noted that patients receiving cremophor EL must be first treated with an antihistamine and an anti-inflammatory agent to prevent anaphylactic shock which has been reported to occur in some patients.

Table 1.9: Parenteral Products Containing Surfactants

TradeName	Active Drug Component	Manufacturer	Route of Administration ^a	% (wlv) Surfactant Composition
M.V.I.	Mu1ti-Vitamin	Armour .	IV Infusion	1-1.7% Polysorbate 20
Loxitane	Loxapine HCI	Lederle	IM	5% Polysorbate 80
VePesid	Etoposide	Bristol .	IV Infusion	8% Polysorbate 80
Amiodarone	Cordaron HCI	Wyeth-Ayersl	IV Infusion	10% Polysorbate 80
Librium	Chlordiazepoxide HCI	Roche	IV (diluent)	4% Polysorbate 80
Taxol	Paclitaxel	BMS ^b	IV Infusion	Cremophor El

^aIV = intravenous, IM = intramuscular. ^bBristol -Myers-Squibb

1.2.5 Solubilization by Complexation

Complexation is the interaction of two species, the solute and the ligand to form a nonbonded identity with a well-defined stoichiometry. Although a wide variety of intermolecular interactions can produce a complex, there are two specific classes useful for increasing the solubility of drugs in aqueous media: stacking and inclusion complexes. Stacking complexes are formed by the overlap of planar regions of aromatic molecules, and inclusion complexes are formed by the insertion of the nonpolar region of one molecule into the cavity of another molecule (or group of molecules).

1.2.5.1 Mathematical representation

The mathematical description for the equilibrium constant of a 1:1 complex is given by Eq. (1),

$$K_{1:1} = [SL]/[S][L]$$
 ...(1)

Where S is the concentration of the free solute, L is the concentration of the free ligand, [SL] is the concentration of the solute-ligand complex, and $K_{1:1}$ is the equilibrium constant also commonly referred to as the stability constant or complexation constant. If two ligand molecules are needed to complex with a solute molecule, the complexation constant is expressed by Eq. (42).

$$K_{1:2} = [SL_2]/[S][L]^2$$
 ...(2).

Likewise other orders can be formed by various combinations of solute-ligand complexes. For a solute in a 1:1 complex system, the total solubility of the solute, S_T , can be simply defined by Eq. (3)

$$S_T = S_w + [SL] \qquad ...(3)$$

Where S_W is the intrinsic solubility of the solute. Similarly, the total concentration of ligand, $[L^{tot}]$, in the system can be given by Eq. (4)

$$[L^{tot}] = [L] + [SL] \qquad \dots (4)$$

Combining Eqs. (1), (3), and (4), gives the general Eq. (5) for solubilization by 1:1 complexation,

$$S_T = S_w + [K_{1:1} S_w L^{tot} / (1 + K_{1:1} S_w)]$$
 ...(5)

Where the intercept is the intrinsic solubility of the solute. Equation (5) represents a linear solubilization of the solute as a function of ligand concentration. The slope, σ_{complex} , can be determined by Eq. (6),

$$\sigma_{\text{complex}} = [K_{1:1} S_w / (1 + K_{1:1} S_w)]$$
 ...(6)

and stability constant is defined by Eq. (7)

$$K_{1:1} = \sigma_{\text{complex}} / [S_w / (1 - \sigma_{\text{complex}})] \qquad ...(7)$$

From the above, it can be seen that, as the stability constant of a 1:1 complex increases, the linear slope increases until the value converges to unity (one ligand molecule solubilize one solute molecule). The linear rise in solubility is a function of ligand concentration (Fig. 1.8). However, the linear solubilization slope does not rise indefinitely. The

complex itself has a given solubility in water, and when this is reached, the total solubility of the solute will plateau. Further addition of the complexing agent can actually result in a decrease in solute solubility.

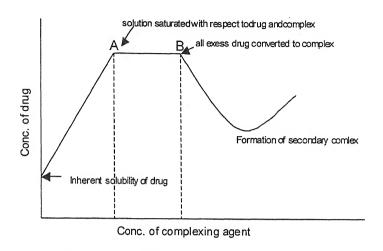


Fig. 1.8: General solubilization profile for complexation

The solubilization curve for a solute molecule that complexes with two ligand molecules is more complicated than that shown in figure 1.8. If the complexation constant for a second ligand is significantly lower than for the first, a 1:1 complex is formed first (i.e., at lower ligand concentrations). It combines with a second ligand to produce a 2:1 complex. Assuming that the latter is more soluble than the 1:1 complex, the solubilization curve has two distinct slopes. If each ligand is equally capable of complexing with the solute, they complex simultaneously, producing a concave-up solubilization curve.

According to Eq. (5), the degree of solubilization of the solute is determined by the complexation constant and the solubilities of the solute and ligand. The solubility of the complex limits the total solubility accordingly. As a result, the most useful ligands for solubilization in aqueous media are highly water soluble, because they tend to produce soluble complexes.

1.2.5.2 Self-association and stacking complexation

All organic drugs placed in water tend to be squeezed out by the strong water-water interactions. This causes some molecules to minimize the contact between their nonpolar hydrocarbon moieties and the polar

1.2.5.3 Inclusion complexes

An inclusion complex is produced by the inclusion of a nonpolar molecule or the nonpolar region of a molecule (known as the guest) into the nonpolar cavity of another molecule or group of molecules (known as the host). When the guest molecule enters the host molecule the contact between water and the nonpolar regions of both is reduced. Thus, inclusion phenomena are the result of the same driving force that produces micellization, self-association, and stacking that is, the squeezing out of nonpolar moities from water.

The major structural requirement for inclusion complexation is the fit of the guest into the host cavity. The most commonly used host molecules are the cyclodextrins (CDs). These cyclic oligomers of glucose are relatively soluble in water and have cavities large enough to accept common nonpolar portions of drug molecules. The naturally occurring cyclodextrins contain 6, 7, and 8 glucopyranose units and are termed α , β and γ , respectively. Some of the more common modifications are done with alkyl or hydroxyalkyl groups or anionic or cationic functionalities. These modifications make cyclodextrins more soluble in water than their naturally occurring precursors ¹³⁸⁻¹⁴⁰.

The size of the cavity of the α , β and γ cyclodextrins is the principal factor in determining which guest solute is most acceptable for complexation (Fig. 1.9). It is also possible to have more than one portion of the molecule complexed by cyclodextrins. Rademacher and Czarnik¹⁴¹

report the complexation of three cyclodextrin molecules to a 1,4,5,8,9, 12-hexaazatriphenylene derivative. In fact, it may be possible to use a combination of cyclodextrins in order to accommodate different functional groups within a given compound. Seo et al. 142 have shown the dependence of the stability constant and overall solubility upon the cyclodextrin ring size for spironolactone. The data demonstrate that for the relatively large spironolactone molecule the stability constant increases with the size of the host cavity, which is reflected by an increase in the linear slopes. The lowest maximum solubility was obtained with β -cyclodextrin, which has the lowest intrinsic solubility of the three cyclodextrins 143.

A significant disadvantage of the naturally occurring cyclodextrins is their limited solubility. For this reason and other physical property limitations, numerous derivatives have been prepared from the cyclodextrins 144-148. One of the most studied of these is hydroxypropyl- β -cyclodextrin, HP- β -CD. This compound is very soluble in water and can be used in high concentrations without intrinsic solubility problems. Pitha et al. 149 investigated high concentrations of HP- β -CD for a variety of compounds and found a wide range of solubility enhancement (Table 1.10). Another modification is substitution of sulfobutyl ether groups (SBE) at various positions of the cyclodextrin structure, which yielded modified cyclodextrins with very high water solubilities. Sulfobutylether β-cyclodextrins, SBE-β-CD, also show good complexation characteristics (Table1.11).

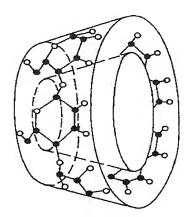


Fig. 1.9: Model of the three dimensional structure of β -cyclodextrins

Table 1.10: Solubility Enhancement by the Use of High concentration of HP-β-CD^a

Solute	% HP-β-CD	Solubility Enhancement ^b
Estriol	50	13,666
Estradiol	40	7000
Progesterone	40	2266
Spironolactone	40	1400 -
Testosterone	40	1461
50% Digoxin	50	971
Dexamethasone	50	240
Chlorthalidone	50	88
Diphenylhydantoin	50	57
Furosemide	50	24
Nitroglycerin	40	8.3
Acctamidophen	50	6
Apomorphine	50	5.8
Theophylline	50	1.3

Table 1.11: Solubility Enhancement by the use of O.1M SBE - β-CD(IV)

Solute	Solubility Enhancement
Testosterone	2020
Prednisolone acetate	426
Dexamethasone	208
Dapsone	189
Prednisolone	106
Methyl prednisolone	89
Hydrocortisone	. 87
Menadione	69
Benzyl guanine	68
Chloramphenicol	8

1.2.2 Solubilization Using a Combination of pH and Complexation

The effect of pH on solubilization by complexation depends entirely on the solute and the ligand. Tinwalla et al.¹⁵⁰ found that the combination of ionization and complexation can be a powerful method for solubilization. Other investigators also found the combination of pH and complexation to be a good method for solubilizing drugs¹⁵¹⁻¹⁵³.

Table 1.12: Parenteral Products Containing Compounds as Excipients that

May Facilitate Complexation

Trade Name	Active Drug Component	Manuf.	Route of Administration ^a	% (w/v) Excipient Composition
Ativan	Lorazepam	Wyeth	IM	2.0% Benzyl alcohol
Dramimine Inj.	Dimenhydrinate	Searle	IM/IV	5.0% Benzyl alcohol
DepoTestostero ne	Testosterone cypionate	Upjohn	IM	10-20% Benzyl benzoate
Metubine Iodide	Metocurine iodide	Eli Lilly	IV	0.5% Phenol
M.V.I.	Multi-Vit	Armour	IV Infusion	2.0% Gentisic acid/ Ethanolamide
Estradurin	Polyestradiol phosphate	Ayerst	IM	2.0% Benzyl alcohol

^aIV = intravenous,JM = intramuscular.

1.2.7 Prodrug

Molecules that contain an alcohol, phenol, carboxylic acid, amine, hydantoin functional group can potentially be derivatized as a prodrug. Once the prodrug is administered in-vivo, the promoiety is hydrolyzed by esterase or phosphatase releasing the parent drug.

1.2.8 Other approaches for formulation of aqueous injections Dispersed System

- (i) Emulsion system: if the molecule has sufficient lipid solubility, it can be formulated for intravenous administration by the use as emulsion. Micro emulsion systems are thermodynamically stable transparent colloidal dispersions.
- (ii) Mixed micelles: Mixed micelles systems are usually composed of two different amphiphilic compounds, usually a phospholipid and a bile salt. The solubilization capacities of mixed micelle formulations have been shown to be dependent on physicochemical factors such as pH, ionic strength, and temperature as well as phospholipid characteristics.
- (iii) Liposomes: A liposome is a lipid bilayer and an aqueous based multilayered spherical drug delivery system where the drug is encapsulated inside the liposome and is released as the liposome is

- eroded in-vivo. Drug candidate for liposome formulas may be water or lipid soluble and usually highly potent.
- Micro and Nanoparticles: Technology of micro and nanoparticle (iv) system is emerging as an alternative to co-solvents for delivery of water insoluble drugs or to provide a sustained release and can be injected intravenously.
- Heroic Approach: This approach describes the efforts to solubilize (v) drugs for early clinical studies, particularly for chemotherapeutic agents, using the additives that probable are not acceptable for commercial formulations. For example, 70% DMSO has used to solubilize an antiviral drug (9-B-D-arabin-furanosyladenine).
- Insoluble Drug Delivery (IDD) Technology: IDD technology is (vi) believed to be the first application of delivering undiluted or highly concentrated drugs substances, micron or sub micron sized particles of the liquid or solid drug stabilized with physiologically safe, tissue compatible and pharmaceutically acceptable surface modifiers such as natural and synthetic lipids.
- (vii) **Dendrimer:** They are well defined, highly branched, macromolecules that radiate from a central core and are synthesized through a stepwise, repetitive reaction sequence that guarantees complete shells for each generation, leading to polymers that are monodisperse.

1.3 INJECTABLE DOSAGE FROM CONSIDERATIONS

Two key aspects of any successful injectable formulation are (i) to achieve the required drug concentration and (ii) drug should be chemically & physically & stable in order to have sufficient shelf life. The ideal injectable formulation from in-vivo tolerability point of view is isotonic with physiological fluids and has a neutral pH 7.4 and thus the formulation scientist must use a wide variety of solubilization techniques. If the stability is insufficient to provide a two-year self-life, then the formulation scientist must change the solution condition to achieve both the solubility and stability.

The intravenous route is the most rapid and most bio-available method of getting a drug into systemic circulation. One of the greatest concerns with the use of solubilized systems is the systemic and local toxicity associated with their administration.

1.4 REVIEW OF LITERATURE

Rytting et al. 154 developed a quantitative structure-property relationship (QSPR) to predict drug solubility in binary mixtures of polyethylene glycol (PEG) 400 and water. The ability of the QSPR model to predict solubility was assessed and compared to the classic log-linear cosolvency model. The QSPR model requires only the chemical structure of the drug and has utility for guiding vehicle identification for early preclinical in vivo studies, especially when compound availability is limited and experimental data such as aqueous solubility and melting point are unknown. When experimental data are available, the log-linear model was verified to be a useful predictive tool.

Nokhodchi et al. 155 studied solubility of four benzodiazepines (BZPs) including diazepam (DIZ), lorazepam (LRZ) clonazepam (CLZ), and chlordiazepoxide (CHZ) in water-cosolvent (ethanol propylene glycol and polyethylene glycol 200) binary systems were. In general, increasing the volume fraction of cosolvents resulted in an increase in the solubility of benzodiazepines. The mole fraction solubilities were fitted to the various cosolvency models, namely extended Hildebrand approach (EHA), excess free energy (EFE), combined nearly ideal binary solvent/Redlich-Kister (CNIBS/R-K), general single model (GSM), mixture response surface (MR-S). double log-log (DL-L), and linear double log-log (LDL-L). The results showed that DL-L model was the best model in predicting the solubility of all drugs in all the water-cosolvent mixtures (OAE% = 4.71).

Martin et al.¹⁵⁶ used the extended Hildebrand solubility approach to estimate the solubility of sulfonamides in binary and ternary solvent systems. The solubility of sulfisomidine in the binary solvent, dioxane-water, shows a bell-shaped profile with a solubility maximum well above the ideal solubility of the drug. This is attributed to solvation of the drug with the dioxane-water solvent, and indicates that the solute-solvent interaction energy (W) is larger than the geometric mean (delta 1 delta 2) of regular solution theory. The solubilities of sulfadiazine, sulfisomidine, sulfathiazole, and sulfamethoxazole were determined in mixtures of dimethylacetamide, glycerol, and water, and the

solubility profiles were well reproduced by use of the extended Hildebrand solubility approach.. None of the four sulfonamides yielded log-linear relationships in the ternary mixtures.

Bustamante et al. 157 studied the behavior of the apparent thermodynamic magnitudes for the solubility of paracetamol, acetanilide, and nalidixic acid. The solubility of these drugs was measured at several temperatures in dioxane-water mixtures. DSC analysis was performed on the original powders and on the solid phases after equilibration with the solvent mixture. The solubility peaks of acetanilide and nalidixic acid shift to a more polar region at the higher temperatures. Nonlinear van't Hoff plots were observed for nalidixic acid whereas acetanilide and paracetamol show linear behavior at the temperature range studied. Two different mechanisms, entropy and enthalpy, are suggested to be the driving forces that increase the solubility of the three drugs. The enthalpy-entropy compensation analysis also suggests that two different mechanisms, dependent on cosolvent ratio, are involved in the solubility enhancement of the three drugs. The plots of deltaH versus deltaG are nonlinear, and the slope changes from positive to negative above 50% dioxane. The study suggested the nonlinear enthalpy-entropy compensation effect may be characteristic of the solubility of semipolar drugs in dioxanewater mixtures.

Yalkowsky and Rubino¹⁵⁸ developed an equation describing solubilization in aqueous systems by cosolvents by treating a mixed solvent as a linear combination of its components. This equation can successfully explain both the exponential increases and the exponential decreases in aqueous solubility that are frequently observed with the addition of cosolvent. It also provides a means of estimating to what extent a particular drug can be solubilized and how much cosolvent would be required to accomplish a particular degree of solubilization.

Yung-Chi et al.¹⁵⁹ proposed an intravenous formulation decision tree for discovery compound formulation development. The proposed decision tree can be adapted and modified by pharmaceutical scientists to conform to best

practices put forth by their institutions for discovery animal studies requiring injectable dosage forms.

Jain et al. 160 investigated solubilization of NSC-639829, an investigational anti-tumor agent, using pH combined with cosolvents, surfactants, and complexants. Solubilization of NSC-639829 was found to be much more effective when the drug was present primarily in ionized form. At pH values 1.0 and 2.0 where the surfactant (SLS) and complexant (SBE2CD) carried a negative charge enhanced solubilities of more than a million-fold were observed for the drug.

Simamora et al.¹⁶¹ presented The solubilization of rapamycin, a poorly water soluble investigational immunosuppressive drug, by facilitated hydrotropy. They reported that partially water-miscible aromatic solutes (such as benzyl alcohol, benzoate, or benzoic acid) could be solubilized by water-miscible cosolvents such as ethanol and propylene glycol. Once solubilized, the partially miscible aromatic solute becomes a solubilizing agent. This technique yielded a dramatic (>1000-fold) increase in the aqueous solubility of rapamycin.

Kerc et al.¹⁶² calculated and experimentally determined the molar solubility of felodipine in water using polyoxyethylene-polyoxypropylene block copolymer (Synperonic T304) and sodium lauryl sulfate (surfactants) and ethanol (cosolvent) as solubilizing agents. Significantly different molar solubilities were determined for felodipine according to the approach used to achieve solubilization. Such differences are the result of the different mechanisms of solubilization of the additives.

Injoon et al.¹⁶³ studied and generated preliminary information on the stability, degradation products, and solubility of oxathiin carboxanilide (a novel inhibitor of HIV which is currently undergoing preclinical evaluation as an anti-AIDS agent) in order to develop prototype parenteral dosage forms to be used in the early stages of preclinical and clinical evaluation. A stability-indicating HPLC assay was developed for use in monitoring drug solubility and stability.

The rate of degradation of oxathiin carboxanilide was determined in aqueous buffers as a function of pH and temperature. The desired solubility was achieved in 70% dimethylacetamide/water and 70–80% dimethylsulfoxide/water cosolvents. A more physiologically compatible extemporaneous lipid emulsion was also prepared containing 0.75 mg/ml of I in Liposyn II 20%. The stability of I in the 20% lipid emulsion was established over a period of 48 h at 25°C.

Rubino and Thomas¹⁶⁴ determined solubilities of the sodium salts of some sulfonamides, barbiturates and hydantoins in mixtures of propylene glycol and water. In many cases, the solubilities of the salts in the mixed solvents were lower than those in water, however, several compounds exhibited enhanced solubilities in the mixed solvents. This unexpected increase in solubility was not related to the lipophilicity of the acidic forms of the drugs and occurred in at least one member of each group of compounds. Analysis of the solid phase which had been equilibrated with each solvent indicated the formation of crystal hydrates for most of the solutes, and in at least one instance, mixed solvates. These compounds could be categorized on the basis of their desolvation temperatures. Those compounds with low temperatures of desolvation had increased solubilities in propylene glycol-water mixtures while compounds with high desolvation temperatures had decreased solubilities in the mixed solvents. These data indicate that crystal hydrate formation plays a significant role in determining if a cosolvent can be used to enhance the solubilities of certain sodium salts.

Sweetana and Akers¹⁶⁵ had briefly reviewed pertinent literature for parenteral drug solubilization and provided tabular information to assist parenteral formulation scientists in tackling and solving their solubility problems.

Jouyban et al.¹⁶⁶ demonstrated that all cosolvency models could be made equivalent by using algebraic manipulations. The models can be divided into two mathematical categories, i.e. linear and non-linear models. Both linear and nonlinear models produced comparable accuracies when an equal number of constant terms was employed in numerical analyses.

He, Li and Yalkowsky¹⁶⁷ evaluated combined effect of cosolvent (methanol (MeOH), ethanol (EtOH), or n-propanol (n-PrOH)) and complexant hydroxypropyl-beta-cyclodextrin (HP beta CD) on the solubility of Fluasterone and explained with a simple equation. The calculated constants in the equation not only quantitatively describe the dependence of drug solubility on cosolvent and ligand concentrations, but also explain the minima that are observed in the Fluasterone solubility versus cosolvent concentration curves at fixed HP beta CD concentrations.

Millard et al. 168 determined the solubilization power (sigma) of the most common pharmaceutical cosolvents: propylene glycol, ethanol, polyethylene glycol 400, and glycerin for a large number of organic compounds from the slope of their log-solubility vs. cosolvent volume fraction plots. The solubilization data at room temperature were either experimentally determined or obtained from the literature. The slopes of the nearly linear relationship between solubilization power and solute hydrophobicity (logK(ow)) were obtained by linear regression analysis for each considered cosolvent. They concluded that knowing or calculating a compound's partition coefficient is all that is needed to predict solubilization.

Ni et al. 169 investigated solubilization of carbendazim by pH in combination with cosolvents, surfactants or complexants. At pH 7 the total drug solubility is 6.11 +/- 0.45 microg/ml which increases by 1-7 fold with cosolvent, surfactant or complexant. However, at pH 2 the solubility increases by 250 times. Cosolvents have a negligible effect (50% increase) on the total drug solubility at pH 2 because of the high polarity of the cationic drug. Also pH combined with nonionic surfactants does not improve solubility, as relatively less polar micelles are not able to accommodate the cationic drug. Interestingly, the total drug solubility increases by combining pH 2 with complexants, as they can form a complex with the isolated aromatic ring of both the unionized and the ionized drug.

Nielsen et al. 170 investigated solubility of bumetanide in vehicles of various polarities, suitable for intranasal administration in acute situations. The

solubility at 4 degrees C in glycofurol and polyethylene glycol 200 was high (167 and 143 mg/mL, respectively), decreasing exponentially with addition of phosphate buffer or coconut oil. Adequate solubility (approximately 50 mg/mL) was achieved in vehicles containing about 80% cosolvent. The stability of bumetanide was studied at 5 degrees C and 57 degrees C. No degradation was observed at low temperature. At high temperature, bumetanide decomposes in nonaqueous vehicles with half-lifes ranging from 69 to 400 days, but sufficient stability may be obtained by adjustment of pH to 7.4. It may be concluded that it is possible to prepare a clinically relevant formulation for intranasal delivery of bumetanide.

studied intensively ternary solvent systems of N,N'-Han et al. 171 dimethylacetamide (DMA)/alcohol/water and Cremophor EL/DMA/alcohol for solubilizing Biphenyl dimethyl dicarboxylate (BDD), a synthetic analogue of schizandrin C, BDD was solubilized effectively in these cosolvents, and the results showed that the cosolvent systems were effective for solubilizing BDD up to the concentration that might be employed for preparation of parenteral dosage forms. Formulation of a BDD concentrate for intravenous infusion was proposed employing the cosolvent system of Cremophor EL/DMA/alcohol.

Tongaree et al. 172 studied solubility of oxytetracycline (OTC) in aqueous and mixed solvent systems. Cosolvent systems of PEG 400, propylene glycol, glycerin, and 2-pyrrolidone were studied in the pH range of 2.5-9. Solubility results showed increased solubility with increased cosolvent concentration, especially in 2-pyrrolidone solvent systems. These results also showed that cosolvents enhanced drug solubility through either their effects on polarity of the solvent medium or complex formation with OTC. Aqueous and mixed solvent systems at lower pH values resulted in higher OTC solubilization because the drug existed primarily in its cationic form.

Li¹⁷³ had developed a simple mathematical model to describe the combined effect of cosolvency and complexation on nonpolar drug solubilization. The total drug solubility is determined by the summation of three drug species present in the solution: free drug [D], drug-ligand binary complex [DL], and drug-ligand-cosolvent ternary complex [DLC]. The proposed model established the dependencies of these three species upon the intrinsic drug solubility, [D(u)], the cosolvent solubilizing power, sigma, the binary and ternary intrinsic complexation constants, K(b)(int) and K(t)(int), and the cosolvent destabilizing powers for the binary and the ternary complexes, rho(b) and rho(t). A nonpolar solute, Fluasterone, is used to evaluate the newly generated equation. The model explains the decline in drug solubility produced by low cosolvent concentrations as well as the increase in the solubility produced by high cosolvent concentrations that are observed at all cyclodextrin concentrations.

Li et al.¹⁷⁴ investigated the roles of both ionized and un-ionized species of flavopiridol in solubilization by complexation, micellization, and cosolvency. Control of pH was used in combination with surfactants (polysorbate 20 and polysorbate 80), cosolvents (ethanol and propylene glycol), as well as uncharged and anionic complexing agents [hydroxypropyl beta-cyclodextrin (HPbetaCD) and sulfobutyl ether beta-cyclodextrin (SBEbetaCD)] to solubilize flavopiridol. These combined techniques increase not only the solubility of the un-ionized flavopiridol but also the solubility of the ionized drug. This study confirms that previously developed equations effectively characterize the roles of pH, pK(a), and either complexation constant, micelle partition coefficient, or cosolvent solubilizing power in determining drug total aqueous solubility.

Raghavan et al.¹⁷⁵ determined solubility of DMP 840 in water, saline, acetate buffers, and cosolvent mixtures and the effect of nicotinamide and pyridoxine concentrations on the solubility of DMP 840 was examined by the phase solubility method. The use of the nontoxic and water-soluble complex-forming agents nicotinamide and pyridoxine resulted in a linear increase in the aqueous solubility of DMP 840 with both ligands. The solubilization appears to be due to formation of 1:1 complexes between DMP 840 and the bioorganic ligands. The NMR results indicate that the interaction is a result of vertical or plane-to-plane stacking and the complexation constants were in agreement with that obtained by phase solubility. The results suggest that the aqueous

solubility of a poorly water soluble drug substance such as DMP 840 can be significantly enhanced by its complexation with water-soluble and nontoxic agents.

Singhai et al.¹⁷⁶ had attempted to increase aqueous solubility of Ketoprofen (1) an analgesic, antipyretic and anti-inflammatory agent, by various cosolvents. The solubility increased up to 8556 times (maximum) in case of ethanol while it was 33 times (minimum) in case of glycerol. Using selected cosolvents and hydrotropes they formulated, aqueous injections of 1.

Tarantino et al. 177 investigated N-Methyl-2-pyrrolidone (methylpyrrolidone) as a cosolvent for model drug compounds of widely varying polarity. These compounds were digoxin, sulfamethoxazole, hydrocortisone acetate, theophylline, phenytoin, and reserpine. Methylpyrrolidone was found to be an extremely efficient cosolvent for low solubility polar drugs such as digoxin or drugs containing multiple proton-donating groups such as phenytoin. Significant deviations from log-linear solubilization were observed with digoxin, sulfamethoxazole, phenytoin, and reserpine, indicating significant water-solute-cosolvent interactions.

Zia et al.¹⁷⁸ studied Dimethyl isosorbide (DMI), an investigational pharmaceutical vehicle, a water-miscible liquid with relatively low viscosity, as a cosolvent for nonpolar drugs and characterized via dielectric constant measurements of binary solvent systems containing DMI and either water, propylene glycol (PG), or polyethylene glycol (PEG). Evidence from the dielectric constant profiles and NMR studies suggested that DMI undergoes complexation with water and PG, but not with PEG, through hydrogen bonding interactions. The solvent complexation exhibited a major effect on the solubilities of prednisone, dexamethasone, and prednisolone in the mixed solvent systems.

Brazeau and Fung¹⁷⁹ explored the influence of organic cosolvent-induced myotoxicity on the bioavailability of a model compound, diazepam. White rabbits were injected with diazepam dissolved in three cosolvent: water

mixtures (20% v/v propylene glycol, 20% v/v polyethylene glycol 400, and 50% v/v polyethylene glycol 400). These mixtures have similar physicochemical properties, but vary 10-fold in their in vitro myotoxicity. The degree of skeletal muscle damage caused by these organic cosolvent systems does not seem to affect the bioavailability of a tracer dose of intramuscular diazepam.

Brazeau and Fung¹⁸⁰ elucidated the mechanisms of organic cosolvents propylene glycol and ethanol induced skeletal muscle damage and creatine kinase release following intramuscular injection.. The study suggested that cosolvent-induced enzyme release from skeletal muscles may be caused by an intracellular mechanism rather than by a direct solubilization of sarcolemma. This intracellular mechanism may involve the mobilization of calcium.

Brazeau and Fung¹⁸¹ examined the potential of binary mixtures of propylene glycol-water, ethanol-water, and polyethylene glycol 400-water to cause skeletal muscle damage (myotoxicity) following intramuscular injection with an in vitro model using the isolated rat muscle. The results suggested that polyethylene glycol 400 in mixed cosolvent systems might have a protective effect on the myotoxicity generated by intramuscular injections.

Rubino and Yalkowsky¹⁸² examined solubilities of three nonpolar drugs, phenytoin, diazepam, and benzocaine, in 14 cosolvent-water binary mixtures and calculated deviations from solubilities by the equation log Sm = f log Sc + (1 - f) log Sw,. Similarities between the results of this study and those of previous investigations suggest that changes in the structure of the solvent play a role in the deviations from the expected solubilities.

Suzuki and Sunada¹⁸³ examined the mechanism for the hydrotropic solubilization of nifedipine, a poorly water-soluble drug, in the aqueous solution of nicotinamide using not only nicotinamide analogues but also urea analogues as aliphatic hydrotropes. The values of stability constants, K1:2, at different temperatures in nicotinamide solution were determined by the phase solubility technique, and were utilized to estimate the thermodynamic parameters of complex formation between nifedipine and nicotinamide. The enthalpy change values suggested the participation of intermolecular forces other than hydrogen bonding in complexion. The aqueous solubility of nifedipine was measured in the presence of nicotinamide, urea and their analogues: N-methylnicotinamide, N,N-diethylnicotinamide, nipecotamide, methylurea, ethylurea and butylurea. The drug solubility increased in proportion to the amount of alkyl substituent on the amide nitrogen, and the solubilizing effect of butylurea was as high as that of nicotinamide. Furthermore, the relationship between the logarithmic drug solubilities in 1.0 M aqueous solutions of nicotinamide or urea analogues versus the logarithmic octanol-water partition coefficient values of ligands as an indication of hydrophobicity was found to be linear. The significant contributor to the hydrotropic solubilization of nifedipine with nicotinamide was therefore the ligand hydrophobicity rather than the aromaticity of the pyridine ring.

Agrawal et al.¹⁸⁴ investigated the effect of various hydrotropes such as nicotinamide, sodium ascorbate, sodium benzoate, sodium salicylate and piperazine on the solubility of nimesulide. The solubility enhancement of nimesulide by the hydrotropes was observed in decreasing order as piperazine > sodium ascorbate > sodium salicylate > sodium benzoate > nicotinamide. They studied various solution properties of hydrotropes such as viscosity, specific gravity, surface tension, refractive index, specific conductance of hydrotropic solutions at 25 +/- 2 degrees C. They concluded that hydrotropic solubilization of nimesulide at lower hydrotrope concentration may be attributed to weak ionic interactions while that at higher hydrotrope concentration may be due to molecular aggregation. They also developed parenteral formulations using piperazine as a hydrotrope and studied for physical and chemical stability.

Gonzalez et al.¹⁸⁵ examined the interaction between a nonionic surfactant (ethoxylated fatty alcohol containing between five and six oxyethylenic units) and sodium p-toluene sulfonate. Surface tension measurements confirm that

the hydrotropic effect occurs at a concentration in which the hydrotropes self-associate. Photon correlation spectroscopy studies showed that for this concentration of hydrotropes a drastic reduction in the surfactant micellar radius occurs. Furthermore the luminescence of the hydrotrope used as a fluorescence probe indicates that at low concentrations p-toluene sulfonate dissolved in the surfactant micelles but beyond the minimum concentration for hydrotropic solubilization the hydrotrope was present in the aqueous phase. These results suggest that the hydrotropic effect is related to alterations in the water structure induced by the hydrotrope molecules and to the presence of hydrotrope aggregates that furnish an appropriate niche for the surfactant amphiphile

Coffman and Kildsig¹⁸⁶ examined the effect of two hydrotropic agents, nicotinamide and urea, on riboflavin solubility in aqueous and nonaqueous systems. The term "solutropy" is introduced to describe solubilization by addition of large amounts of a second solute in any solvent. The nonaqueous solvents used included methanol, N-methylformamide, dimethyl sulfoxide, and acetone. In water, methanol, and N-methylformamide, riboflavin solubility was found to increase with increasing nicotinamide concentration; however, riboflavin solubility decreased with increasing nicotinamide concentration in dimethyl sulfoxide and acetone, thus establishing the solvent-dependent nature of solutropy. An examination of solvent properties revealed that the solvent's ability to be both a proton donor and acceptor is important mechanistically, while dielectric constant and polarity are not. The same solvent-dependency was observed with urea, although urea is a poorer solutrope than nicotinamide. This study proposes that some solutropic agents act by changing the nature of the solvent, specifically by altering the solvent's ability to participate in structure formation via intermolecular hydrogen bonding.

Muller and Albers¹⁸⁷ investigated influence of hydrotropic compounds on complex formation by 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD) with methyltestosterone (MeT). Various representatives of the lyotropic series

were used for this purpose. Additive hydrotropic effects were observed for nicotinamide and urea, which disrupt the water structure, while structure formers such as sorbitol exerted negative effects. The effects of hydrotropic substances on the phase solubility relationship of MeT showed that inclusion complex formation with 2-HP-beta-CD depends on the degree of ordering of the solvent and is apparently subject to entropy effects. Combined systems comprising 2-HP-beta-CD and auxiliary substances with various underlying solubilizing principles were also investigated. Combination of 2-HP-beta-CD with conventional solubilizers, such as 1,2-propylene glycol or sodium deoxycholate, reduced the solubilization capacity of 2-HP-beta-CD. Competitive displacement of the inclusion molecule from its 2-HP-beta-CD complex by sodium deoxycholate suggested that cholesterol participates in the release mechanism of the inclusion molecule under in vivo conditions. The spontaneous release of complexed drug molecules could indirectly be shown on the basis of the spontaneous action of a complexed dihydropyridine derivative after iv administration in rats. The bioavailability of an investigational drug in cynomolgus monkeys could be enhanced sevenfold by inclusion complexation with 2-HP-beta-CD.

Saleh et al. 188 investigated effect of some electrolytes, nonelectrolytes, surfactants, and hydrotropic salts on the solubility of water in 1-butanol and 1-hexanol. While sodium chloride and sodium acetate decrease the solubility of water in 1-butanol, urea has no significant effect. The ionic surfactants, sodium lauryl sulfate and cetrimide, cause an initial decrease in the solubility of water in 1-butanol followed by an increase at high surfactant concentrations. The nonionic surfactant, polysorbate 20, does not show the initial decrease in water solubility. On the other hand, the hydrotropic salts, sodium benzoate, sodium salicylate, and sodium gentisate, are shown to be the best water solubilizers in 1-butanol. Sodium salicylate showed the maximum solubilizing power. The effect of sodium benzoate, sodium salicylate, and sodium lauryl sulfate on the solubility of water in 1-hexanol was also investigated. Similar results were obtained.

Lee et al. 189 identified several hydrotropic agents effective for increasing aqueous solubility of paclitaxel and analyzed the structural requirements for this hydrotropic property. This information can be used to find other hydrotropic compounds and to synthesize polymeric hydrotropes that are effective for paclitaxel and other poorly water-soluble drugs.

Coffman and Kildsig¹⁹⁰ examined the mechanism of hydrotropic solubilization using the riboflavin-nicotinamide system. They concluded that complexation of nicotinamide and riboflavin does not occur. However, since increasing temperature causes a decrease in the hydrotropic ability of nicotinamide and in its degree of self-association, it is proposed here that the self-association of nicotinamide impacts the hydrotropic mechanism.

Jain et al. 191 had attempted to formulate a stable, aqueous injection of nifedipine (1) using the technique of hydrotropic solubilization. Sodium benzoate (2; 30% w/v) and sodium salicylate (3; 30% w/v) have been employed as the vehicle to prepare the injection of 1 in 1 mg/5 ml and 1 mg/2 ml concentration in either case. An accelerated stability study for 4 weeks at 8 degrees C, room temperature, 40 and 50 degrees C indicates maximum stability at 8 degrees C, suggesting the need of storage with refrigeration. Light stability study in the specially designed chamber indicates that covering the ampoules with black chart paper affords adequate stability to the 1 injection. The injections are also stable to autoclaving. Preliminary in vivo study in rats showed that the injection is effective.

Jain et al. 192 solubilized Nifedipine (1), a practically water insoluble drug, employing sodium benzoate (2) and sodium salicylate (3) as hydrotropes. In 30% w/v 2 solution the solubility of 1 increased 85 and 76 fold at 25 +/- 1 degree C and at 37 +/- 1 degree C respectively. The corresponding increase in solubility of 1 in 30% w/v 3 solution was 135 and 107 fold respectively. To study the mechanism of hydrotropic solubilization of 1, the solution properties of 2 and 3 over a concentration range of 1 to 30% w/v were undertaken. The probable mechanism involves a complexation type of interaction at a low concentration of hydrotrope, aggregation of the hydrotropic molecules and

inclusion of 1 in these aggregates at high concentration; and structural changes in water caused by hydrotropes.

John¹⁹³ Delaney discussed different methods to predict the solubility of a compound in water, together with the issues that affect their applicability.

Backensfeld et al. 194 investigated the effects of hydroxyalkylated cyclodextrins (CD) on the solubility and stability of the nonsteroidal antirheumatics (NSA) diclofenac, piroxicam and indomethacin. The influence of 2-cyclodextrin (2-CD) and HP-2-CD has the most stabilizing effect for diclofenac and indomethacin. In contrast to indomethacin and diclofenac, the CD have a destabilizing effect on the stability of piroxicam.

Fini et al.¹⁹⁵ had analyzed salt forms of ten non-steroidal anti-inflammatory drugs (NSAID) of the acetic and propionic classes to disclose the solubilization property of their aqueous solutions toward a lipid probe (the azodye Orange OT). This property was related to the self-aggregation of the anions above a concentration value that differs for each drug. They briefly discussed the results in terms of hydrophilic/hydrophobic balance present on the anion of the NSAID examined, as evaluated by the fragment constant approach.

Muller and Brauns¹⁹⁶ studied and tested solubilizing properties of Alkyl hydroxy derivatives of 2-cyclodextrin by the solubility method in combination with hydrocortisone, diazepam, digitoxin and indomethacin. Because of the high water solubility of the derivatives, as well as of the formed complexes, it was shown possible to use them for the solubilization of different pharmaceuticals. In addition, a stabilizing effect of a derivative on aqueous solutions of indomethacin was also observed.

Najib and Suleiman¹⁹⁷ examined the effect of two surfactants: sodium lauryl sulphate (SLS) and polysorbate 80, and two hydrophilic polymers: polyethylene glycol (PEG 6000) and polyvinylpyrrolidone (PVP) on the aqueous solubility of indomethacin. It is found that all the above solubilizing

agents increase the solubility of the drug in the following order: SLS > polysorbate 80 > PEG 6000 > PVP. The work also includes calculation of some thermodynamic functions such as the heat of solution (ĆH) and the free energy change (ĆG). In addition the solvent power of the PEG 400/water system is determined. The number of indomethacin molecules bound to a molecule of PEG is calculated and an equation describing this binding process is proposed.

Bogdanova et al.¹⁹⁸ prepared a series of indomethacin/nicotinamide binary system-melts and studied some aspects of the potential physico-chemical interactions between indomethacin and nicotinamide. The comparative analysis of the data from FT-IR- and UV spectroscopy, DSC, X-ray diffractometry and equilibrium solubility study showed that formation of complexes of different stoichiometry could take place in the solid state and in solution.

Casella et al. 199 assessed the solubility, dissolution behavior, complex-binding constant, crystallinity and enthalpy of five unique complexes containing 2-cyclodextrin, indomethacin, ammonia and water. The results show indomethacin solubility was improved by complex formation with 2-cyclodextrin. The complex-binding constants were found to support a theory reported previously that 2-cyclodextrin ring cavity solvation was the predominant factor responsible for complex formation.

Attwood et al.²⁰⁰ investigated the effect of indomethacin on the micellar properties of the non-ionic surfactant, polysorbate 80, in water-sorbitol mixtures containing up to 25% w/v sorbitol by light scattering, photon correlation spectroscopy and viscometric techniques. They found that the micelles in all systems were most satisfactorily represented as oblate ellipsoids, the asymmetry and hydration of which increased with increase of sorbitol concentration. Indomethacin solubilization caused a restructuring of the micelle to produce a more symmetrical micelle of increased hydration.

Pawelczyk and Knitter²⁰¹ worked out 3% aqueous indometacin solution by solubilization of indometacin by means of ethyl carbamate and ethylurea in the concentration of 30% each and boiling the mixture for 30 s. The stability of indometacin in the above solution was checked with respect to elevated temperatures and exaggerated UV light conditions and compared with the stability of the same solution but without the solubilizing agents. It appeared that the solubilizing agents diminish both the hydrolytic as well as the photochemical degradation processes of indometacin. 3% aqueous indometacin solution is stable for 25 months at 20 degrees C, and it is practically non-susceptible to the effects of diffused light for many years.

El-Sabbagh et al.²⁰² employed Non-ionic surface-active agents such as Tween 80 and 40, Myrj 53 and Brij 99,--urea and sodium citrate--urea-indomethacin coprecipitate for the solubilization of indomethacin (an antirheumatic drug). The solubility of indomethacin by nonionic surface active agents was in the following order: Tween 80 and Brij 99 greater than Tween 40 greater than Myrj 53. The efficiency of solubilization by these surfactants decreased as the polyoxyethylene chain increased. Urea increases the solubility of indomethacin due to enhancing the solvation of the drug by water. Up to a concentration of 0.2 M sodium citrate increases the solubility of indomethacin, followed by a sharp decline in the solubility by increasing its concentration of sodium citrate. Urea-indomethacin coprecipitate show slight increase in the solubility of the drug.

Chen et al.²⁰³ developed a quantitative structure-property relationship (QSPR) for predicting the aqueous solubility of drug-like compounds from their chemical structures. A set of 321 structurally diverse drugs or related compounds, with their intrinsic aqueous solubility collected from literature, was used in this analysis. A series of molecular descriptors was calculated directly from chemical structures and a set of eight descriptors, including dipole moment, surface area, volume, molecular weight, number of rotatable bonds/total bonds, number of hydrogen-bond acceptors, number of hydrogen-bond donors and density, was chosen for the final model. The model has a

correlation coefficient (r) of 0.95 and a root-mean-square (rms) error of 0.56 log unit. It predicts the solubility of testing set compounds with a reasonable degree of accuracy (r = 0.84 and rms = 0.86 log unit).

Chen and Frank²⁰⁴ made comparisons of the spectra of chlorzoxazone before and after solubilization in solutions of Tween 80, sodium lauryl sulfate, and cetyltrimethylammonium bromide (CTAB), and found most significant changes for the cationic CTAB. Subsequent studies of the interaction of chlorzoxazone with CTAB indicated that the quaternary ammonium group induces the ionization of chlorzoxazone from its nonionic keto form to its ionized enolate form.

Lin et al. 205 prepared different complexes of indomethacin and both β -cyclodextrin (2CD) and hydroxypropyl 2-cyclodextrin (HP2CD) using different methods: kneading, spray-drying and neutralization followed by freeze-drying. The complexes obtained were studied in the solid phase by differential scanning calorimetry (DSC), thermomicroscopy (TM), and infrared spectroscopy (IR), and in the liquid phase by 1H-NMR. The results showed that the nature of the end products depends on the method of preparation. The neutralization technique led to a true inclusion of sodium indomethacin in the cyclodextrin cavity, while the nature of the spray-dried product, indomethacin (acid form)/cyclodextrin, was not well defined. The kneading method did not lead to a real inclusion. In any case, the complexes obtained may be of great value as rapidly dissolving forms of indomethacin in water.

1.5 RESEARCH ENVISAGED AND PLAN OF WORK

Solubility of organic compounds is in general of Pharmaceutical interest because it has been recognized as a key factors in pharmacological profile of a drug, its chemical stability and ultimately its formulation.

Drug solubilization has been a subject of many scientific articles and textbooks; yet despite this attention and available literature, product development scientist still encounters significant difficulties in their solubility problem. Theories of solute solubilization are not easy to understand. Solubilization processes are amazing, complex and require a fair amount of expertise in physical chemistry to interpret and apply current theoretical models.

If an insoluble drug is to be administered parentally or as an oral liquid it is necessary to find a mean of increasing its aqueous solubility. Parenteral formulations are highly useful in various diseased conditions in which oral administration is contra indicated, quick action is required or dose reduction is advised. In most of the cases, it is not possible to provide quick relief to a patient because of non-availability of aqueous injection of various drugs. Although there has been a critical need for such products yet surprisingly not much are available even in the international market.

The present project is planned to study and discuss the solubilization behavior of **chlorzoxazone**, a centrally acting muscle relaxant and **indomethacin**, a non-selective cox-inhibitor in cosolvents, hydrotropes, complexant, surface-active agents and their combinations and explore their use in parenteral formulations. Further, The study is planned to formulate the selected drugs in solubilized form for injectable use that minimize risk and allow the formulation more stable, economic, safe, effective and, therapeutically significant.

PLAN OF WORK

The proposed work may proceed along the following steps:

- 1. Selection of drug(s) and components of solubilized system.
- 2. Preformulation studies of drug (s)
 - (i) Identification and purity of drug (s)
 - (ii) Differential scanning calorimetry of drug (s)
 - (iii) Hydrolysis profile of drug (s) under ambient conditions.
 - (iv) Solubility studies at various pH
- 3. Solubilization of drug(s)
 - (i) Hydrotropic solubilization
 - (ii) Complexation solubilization
 - (iii) Co-solvent solubilization
 - (iv) Surfactant solubilization
 - (v) Mathematical analysis of solubility data
- 4. Development of aqueous injection formulation
 - (i) Selection of solubilizer
 - (ii) Preparation of Prototype formulation
 - (iii) Characterization of formulations
 - (iv) Stability studies
- 5. In-vivo performance of drug formulation
- (i) Periodical monitoring the drug plasma level
- 6. Compilation, analysis and interpretation of results.

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Drugs and Solubilizers

Chlorzoxazone¹⁻⁸

Category

Skeletal muscle relaxant

Molecular Weight

169.565 g/mol

Molecular Formula

C₇H₄CINO₂

Melting point

191.5 °C range 189 °C- 194 °C

PKa

8.3

pH of suspension

6.5

IUPAC Name

5-chloro-3H-benzooxazol-2-one

History

The skeletal muscle relaxant properties of Chlorzoxazone were first discovered by March at Mc Neil laboratories in the late 1950.

Description

Chlorzoxazone is a benzoxazole derivative and a member of centrally acting muscle relaxant.

Dose

250-750 mg orally three or four times dally.

Physical Appearance

Colorless crystals or white to creamy white crystalline powder, odorless with a bitter taste.

Solubility

Sparingly soluble in water; soluble in methanol, ethanol, isopropanol; freely soluble in aqueous solution of alkali hydroxides and ammonia.

Stability

Chlorzoxazone is stable in the solid state for up to 5 year at room temperature. An aqueous suspension of the drug in distilled water or acidic media is generally stable but in solution in pH greater than 7, the solution is generally not stable.

Clinical Pharmacology

Chlorzoxazone is a centrally acting central muscle relaxant with sedative properties. It is claimed to inhibit muscle spasm by exerting an effect primarily at the level of the spinal cord and subcortical areas of the brain. Data available from animal experiments as well as human study indicate that chlorzoxazone acts primarily at the level of the spinal cord and subcortical areas of the brain where it inhibits multisynaptic reflex a.c. involved in producing and maintaining skeletal muscle spasm of varied etiology. The clinical result is a reduction of the skeletal muscle spasm with relief of pain and increased mobility of the involved muscles. Chlorzoxazone is devoid of uricosuric activity it is apparently less toxic to the liver. Chlorzoxazone inhabit granulation of mast cells subsequently preventing the release of Histamine and slow reacting substance of anaphylaxis (SRS-A) mediators of type 1 allergic reactions. Chlorzoxazone may also reduce the release of inflammatory leukotrienes. Chlorzoxazone may act by inhibiting calcium reflux.

Pharmacokinetics

Blood levels of chlorzoxazone can be detected in people during the first 30 minutes and peak levels may be reached, in the majority of the subjects, in about 1 to 2 hours after oral administration of chlorzoxazone. Chlorzoxazone is rapidly metabolized and is excreted in the urine, primarily in a conjugated form as the glucuronide. Less than one percent of a dose of chlorzoxazone is excreted unchanged in the urine in 24 hours.

Protein binding: 13-18%

Indications

Chlorzoxazone is indicated for relief of discomfort associated with severe painful muscles spasm associated with musculoskeletal disorders such as fibrositis, bursitis, myositis, spondylitis, sprains and muscles strains. It is also indicated as an adjunct to rest, physical therapy, and other measures for the relief of the discomfort

Chlorzoxazone a synthetic compound inhibit antigen-induced bronchospasm and hence is used to treat asthma and allergic rhinitis.

Chlorzoxazone is used as ophthalmic solution to treat conjunctivitis and is taken orally to treat systemic mastocytosis and ulcerative colitis.

Side Effects

Chlorzoxazone containing products are usually well tolerated. It is possible in rare instances that chlorzoxazone may have been associated with gastrointestinal bleeding. Drowsiness, dizziness, lightheadedness, malaise, or overstimulation may be noted by an occasional patient. Rarely, allergic-type skin rashes, petechiae, or ecchymoses may develop during treatment. Angioneurotic edema or anaphylactic reactions are extremely rare. There is no evidence that the drug will cause renal damage. Rarely, a patient may note discoloration of the urine resulting from a phenolic metabolite of chlorzoxazone. This finding is of no known clinical significance.

Drug Interactions

Warnings

Serious (including fatal) hepatocellular toxicity has been reported rarely in patients receiving chlorzoxazone. The mechanism is unknown but appears to be idiosyncratic and unpredictable. Factors predisposing patients to this rare event are not known. Patients should be instructed to report early signs and/or symptoms of heptatoxicity such as fever, rash, anorexia, nausea, vomiting, fatigue, right upper quadrant pain, dark urine, or jaundice. Chlorzoxazone should be discontinued immediately and a physician consulted if any of these signs or symptoms develop. Chlorzoxazone use should also be discontinued if a patient develps abnormal liver enymes (e.g. AST, ALT, alkaline phosphatase, and bilirubin).

The concomitant use of alcohol or other central nervous system depressants may have an additive effect.

Usage in Pregnancy: The safe use of chlorzoxazone has not been established with respect to the possible adverse effects upon fetal development. Therefore, it should be used in women of childbearing potential only when, in the judgment of the physician, the potential benefits outweigh the possible risks.

Precautions

Chlorzoxazone should be used with caution in patients with known allergies or with a history of allergic reactions to drugs. If a sensitivity reaction occurs such as urticaria, redness, or itching of the skin, the drug should be stopped.

If any symptoms suggestive of liver dysfunction are observed, the drug should be discontinued.

Overdose

Symptoms

Initially, gastrointestinal disturbances such as nausea, vomiting, or diarrhea together with drowsiness, dizziness, lightheadedness or headache may occur. Early in the course there may be malaise or sluggishness followed by marked loss of muscle tone, making voluntary movement impossible. The deep tendon reflexes may be decreased or absent. The sensorium remains intact, and there is no peripheral loss of sensation. Respiratory depression may occur with rapid, irregular respiration and intercostal and substernal retraction. The blood pressure is lowered, but shock has not been observed.

Treatment

Gastric lavage or induction of emesis should be carried out, followed by administration of activated charcoal. Thereafter, treatment is entirely supportive. If respirations are depressed, oxygen and artificial respiration should be employed and a patent airway assured by use of an oropharyngeal airway or endotracheal tube. Hypotension may be counteracted by use of dextran, plasma, concentrated albumin or a vasopressor agent such as norepinephrine. Cholinergic drugs or analeptic drugs are of no value and should not be used.

Contraindications

Chlorzoxazone is contraindicated in patients with known intolerance to the drug.

Dosage and Administration

Usual Adult Dosage: One caplet three or four times daily. If adequate response is not obtained with this dose, it may be increased to 11/2 caplets (750 mg) three or four times daily. As improvement occurs dosage can usually be reduced.

Dosage Form

- Chlorzoxazone 500 mg caplets, (capsule shaped tablet
- Dispense in tight container as defined in the official compendium.
- Store at controlled room temperature (15°-30°C, 59°-86° F).

ANALYTICAL METHODS OF CHLORZOXAZONE

Ultraviolet Spectrophotometry

Kale et al.⁹ developed a simple accurate method for simultaneous estimation of chlorzoxazone and nimesulide from combined dosage form. The method was based on the UV absorbance maxima in 0.1 N NaOH. Chlorzoxazone showed two maxima at 243 and 255 nm While nimesulide at 393 nm both drog obeyed Beer's Law in the conc. Range 0.2–40 ng/ml.

Bhatia and Dhaneshwar¹⁰ had developed a simple rapid method for simultaneous estimation of diclofenac sodium, chlorzoxazone and Paracetamol in three component table formulations. The method was based on the native Ultraviolet absorbance maximas of the three drugs in 0.02 N sodium hydroxide.

Conney et al.¹¹ utilized a back-extraction approach to quantitate chlorzoxazone at 289 nm. The authors also reported the extraction and estimation of chlorzoxazone in urine. Petroleum ether containing 1.5% iso-amyl alcohol was shaken with the urine sample for 60 minutes. The organic phase was separated and the drug was back extracted into 0.5N sodium hydroxide. Absorbance read at 289 nm followed Beers Law. Recovery of drug from urine was 93±2%.

Kirschner¹² reported that chlorzoxazone could directly determined at 282 nm in absolute methanol. The authors also reported an assay for the drug in tablets by concomitantly determining the absorbances of the Assay and standard preparations at 282 nm, with a suitable spectrophotometer, using methanol as the blank.

Bhatia et al.¹³ have reported two methods for estimation of chlorzoxazone and paracetamol in two component tablet formulation. The methods employed first derivative UV spectrophotometry and simultaneous equations for the simultaneous estimation of the two drugs at the absorption maxima 244 nm & 255 nm for chlorzoxazone and 257 nm for Paracetamol in 0.02 M Sodium hydroxide.

Ellaithy et al. 14 developed Ratio spectra first derivative spectrophotometry (DR1) for monitoring the change in chlorzoxazone concentration during the degradation process. 2-Amino-4-chlorophenol was found to be the alkaline induced degradation product and the synthetic precursor of chlorzoxazone. Chlorzoxazone was found to follow pseudofirst order kinetics. Kinetic parameters (rate constant (K) and half-life ($t_{0.5}$)) were calculated at different temperatures ($t_{0.5}$) and different sodium hydroxide concentrations ($t_{0.5}$). Activation energy at 3 and 8 M sodium hydroxide concentration and alkaline induced catalysis constant at 60, 70 and 80°C were also calculated.

lodometric Titrimetry

Beral et al.¹⁵ titrated chlorzoxazone (after treatement with boiling 10% sulfuric acid, potassium bromide and potassium bromate) with 0.1 N sodium thiosulphate after potassium iodide was added to cooled solution.

Gas Chromatography

Avadhanulu et al.¹⁶ had reported a simple, fast accurate and precise gas chromatographic method for estimation of chlorzoxazone and paracetamol in single and combined dosage forms using 10% OV-17 column and flame ionization detector; phenacetin was used as internal standard.

Kaempe¹⁷ has charomatographed chlorzoxazone on a packed 15% Dexsil 300 on HP Chromosorb w 80/100 mesh glass column (6ft X 6.4 mm i.d.). The retention time was 0.62 relative to caffeine using a column temperature of 220°C.

Parker et al. 18 attempted to chromatograph the drug on SE-30 (0.05% on glass microbeads 60/80 mesh) at 150 and 165°C column temperature with no success.

Desiraju et al. ¹⁹ chromatographed chlorzoxazone on a 6 ft X 4 mm i.d. glass column packed with 3% OV-1 on 60/80 mesh Gas Chrom Q (14). Column temperature was 130°C and retention time was 3.25 minutes using a column flow rate of 30 ml/minute. The authors also reported a method for the quantitation of chlorzoxazone in plasma samples. The drug is extracted from acidified plasma with ethyl acetate containing the internal standard, n-hexadecane. The ethyl acetate is separated and evaporated to dryness. Pyridine and acetic anhydride are added to the residue, the tube is capped, and the reaction allowed to proceed at 42°C for 20 minutes. An aliquot of the mixture is injected into the gas chromatograph.

Beckett et al.²⁰ reported that chlorzoxazone can be chromatographed on a 2.5% SE- 30 on 80/100 mesh chromosorb W acid-washed HMDS treated 5ftX4mm i.d. glass column at a column temperature of 225°C. Retention time was 0.69 relative to diphenhydramine. Nitrogen was used as carrier gas at 50 ml/min and FID detection was utilized.

Pedroso and Moraes²¹ have chromatographed chlorzoxazone on 2.5% SE-30 and 3% OV-17 at 200 and 220°C column temperature, respectively. They used the retention data to perform qualitative analysis of the drug based on Kovat's Retention indices.

Paper Chromatography

Clarke²² reported that chlorzoxazone gave an Rf of 0.93 using a mobile phase of 87:13 n-butanol-water containing 0.48% citric acid.

Thin Layer Chromatography

El-Bayoumi et al.²³ described a quantitative thin layer procedure for estimating primidone, clorazepate dipotassium and chlorzoxazone in bulk powders and in dosage forms, each in the presence of its degradation product. The method consisted of dissolving the drug in ethanol (for primidone), or methanol (for clorazepate dipotassium and chlorzoxazone) and then spotting this solution on a thin layer of silica gel G254. Quantitation was achieved by comparing the areas under the peaks obtained from scanning the thin layer chromatographic plates in a spectrodensitometer

Ismat Ullah et al.²⁴ developed method for Determination of degradation kinetics of chlorzoxazone by thin-layer chromatography .

High Performance Liquid Chormatography

Honigberg et al.²⁵ have chromatographed chlorzoxazone on an octadecylsilane column using varying proportions of absolute methanol-distilled water. Chlorzoxazone was effectively separated from acetaminophen in a mixture with 50:50 methanol-water at a 2.0 ml/min flow rate using refractive index detection. The authors also reported assay for chlorzoxazone in plasma based on ether extraction of the compounds form acidic plasma. The separation was achieved on an octadecylsilane column (30cm x 3.9 mm i.d.) at a flow rate of 2 ml/min with UV detection at 280 nm and a mobile phase of 40:60absolute methanol-distilled water. Retention times for the hydroxy compound and drug were approximately 4 and 8 minutes, respectively. The limit of detection for each compound was calculated to be 80 ng at singal / noise = 2.

Stewart and Carter²⁶ reported modifications in the above-mentioned HPLC assay by, Honigberg et al in which an octadecylsilane solid-phase extraction column was used to separate chlorzoxazone and 6-hydroxychlorzoxazone from human serum. Percent recoveries were 90.24±4.28 and 86.06±3.58% for drug and metabolite, respectively. The separation was achieved on an octadecylsilane column using a mobile phase of 50:50 acetonitrile–aqueous 0.05M sodium dihydrogen phosphate, pH4.5

with phenacetin as internal standard. The two compounds were detected at the glass carbon electrode using a cell potential of + 1300 mV. These modifications halved the original HPLC analysis time and increased detection for each compound to 2.5 ng, 30 times the previous limit of detection using 280 nm.

A gradient HPLC method for chlorzoxazone with Acetaminophen tablets is given in USP (XXIII)²⁷. In the method, m-Chloroanilline, p-aminophenol (a degradation product of acetaminophen), p-chlorophenol and 2-amino-4-chlorophenol are separated from chlorzoxazone, acetaminophen and the internal standard phenacetin.

Pant et al.²⁸ have demonstrated a simple HPLC method for the simultaneous determination of oxyphenbutazone, chlorzoxazone & paracetamol using caffeine as an internal standard. The assay was carried out using mu Bondapack (C18) column and methanol. ammonia (40 : 60 : 0.1) as mobile phase. The elute monitored with a 240 nm UV detector.

Non-Aqueous Titrimetric

A non aqueous titrimetric method for estimation of chlorzoxazone is given in Japan Pharmacopoeia²⁹. The titration was made with 0.1 N sodium hydroxide in dimethylformamide using thymol blue as indicator.

Schekenburger and Guade-Henkel³⁰ reported titration of chlorzoxazone in dimethylsulphoxide using 0.1N propanolic potassium hydroxide as titrant and metanil yellow as visual indicator.

Colorimetry

Sastry and Chintalapati³¹ developed three sensitive spectrophotometric methods in visible region for determination of chlorzoxazone. In all the three methods drug was treated with an excess of oxidant [Nitrous oxide in method A; N-Bromo sccinimides in method B, Chloramine T in method C] in acidic medium. The untreated oxidant was then estimated colorimetrically by using an oxidisable dye [cresyl for violet acetate, blue and gallocyanine respectively in methods A B & C.] All the methods A, B, and C followed beer's Law in conc. range of 0.4 to 4 : g /ml, 0.4 - 5.0 : g /ml & 2 - 12 : g /ml respectively.

Sanghoi³² reported a colorimetric procedure for chlorzoxazone based on base hydrolysis of the drug followed by diazotization of the product with nitrous acid formed in situ. The color measured at 405 nm is stable for 60 minutes and obeys Beer's Law in the 4-32 ng/ml range. The author also reported estimation of chlorzoxazone in tablets. Acetaminophen and indomethacin were shown to interfere with the method. Recovery of chlorzoxazone in bulk powder and tablet samples was in the 98.3-99.9% range utilizing the assay procedure.

Polarizing Microscopy

Watanbe et al.³³ reported that polarizing microscopy can be used as a qualitative identification tool for chlorzoxazone.

Potentiometry

Tagami and Muramoto³⁴ described a potentiometric method for the determination of chlorzoxazone, based on the use of a carbon dioxide gas-sensing electrode. Upon refluxing the drug with 3N sodium hydroxide, aminophenol and sodium carbonate are formed. Acidification of the reaction mixture yields carbon dioxide, which is sensed by the gas-permeable membrane electrode. A linear calibration plot was obtained within the 3X10⁻⁴ to 5 x 10^{-3} M range of Chlorzoxazone. The authors also described a method for determination of chlorzoxazone in tablet dosage form. Twenty tablets were powdered and a portion equivalent to about 424 mg of drug was weighed and extracted with 20 ml of acetone. After stirring and centrifugation, the supernatant acetone solution was removed and diluted with additional acetone. This extraction procedure was performed four times. The collected acetone fractions were evaporated to dryness. The residue was refluxed for 2 hr with 50 ml of 3N sodium hydroxide and after acidification; the carbon dioxide was determined using a gas permeable electrode. Mean recovery of chlorzoxazone was 99.6% with a standard deviation of 0.24.

Indomethacin³⁵⁻⁵¹

Category

Anti-inflammatory; analgesic.

Molecular Formula

C₁₉H₁₆CINO₄

Molecular Weight

357.79

Melting point

158°C to 162°C.

PKa

4.5

pH of suspension

4.0 - 5.0

History

$$\begin{array}{c|c} & & & \\ &$$

IUPAC Name 1-(4-chlorobenzoyl)-5-methoxy-2methylindol-3-ylacetic acid.

Indomethacin was discovered in 1963 and it was first approved for use in the U.S. by the Food and Drug Administration in 1965. Its mechanism of action, along with several other NSAIDs that inhibit COX, was described in 1971.

Description

Indomethacin is a methylated indole derivative and a member of the arylalkanoic acid class of NSAIDs.

Dose

Orally, 50 to 200 mg daily, in divided doses, with food. As suppositories, 100 mg at night and in the morning if required. Maximum combined oral and rectal dose, 150 to 200 mg daily.

Physical Appearance

Indomethacin is white to pale yellow, crystalline powder; odorless or almost odorless

Solubility

Indomethacin is soluble in chloroform; sparingly soluble in ethanol (95%) and in ether, it is practically insoluble in water.

Stability

It is stable in neutral or slightly acidic media and decomposes in strong alkali.

Clinical Pharmacology

Indomethacin is a non-steroidal drug with anti-inflammatory, antipyretic and analgesic properties. Indomethacin is a nonselective inhibitor of cyclooxygenase (COX) 1 and 2, enzymes that participate in prostaglandin synthesis from arachidonic acid. Prostaglandins are hormone-like molecules normally found in the body, where they have a wide variety of effects, some of which lead to pain, fever, and inflammation. Indomethacin is a potent inhibitor of prostaglandin synthesis in vitro. Concentrations are reached during therapy, which have been demonstrated to have an effect in vivo as well. Prostaglandins sensitize afferent nerves and potentiate the action of bradykinin in inducing pain in animal models. Moreover, prostaglandins are known to be among the mediators of inflammation. Since indomethacin is an inhibitor of prostaglandin synthesis, its mode of action may be due to a decrease of prostaglandins in peripheral tissues.

Indomethacin suppresses inflammation in rheumatoid arthritis as demonstrated by relief of pain, and reduction of fever, swelling and tenderness. Improvement in patients treated with Indomethacin for rheumatoid arthritis has been demonstrated by a reduction in joint swelling, average number of joints involved, and morning stiffness; by increased mobility as demonstrated by a decrease in walking time; and by improved functional capability as demonstrated by an increase in grip strength.

Prostaglandins also cause uterine contractions in pregnant women. Indomethacin is an effective tocolytic agent, able to delay premature labor by reducing uterine contractions through inhibition of PG synthesis in the uterus and possibly through calcium channel blockade.

Indomethacin has two additional modes of actions with clinical importance: It inhibits motility of polymorphonuclear leucocytes, like colchicine. It uncouples oxidative phosphorylation in cartilaginous (and hepatic) mitochondria, like salicylates. These additional effects account as well for the analgesic and the antiinflammative properties.

Indomethacin easily crosses the placenta, and can reduce fetal urine production to treat polyhydramnios. It does so by reducing renal blood flow

and increasing renal vascular resistance, possibly by enhancing the effects of vasopressin on the fetal kidneys.

Indomethacin has been reported to diminish basal and CO₂ stimulated cerebral blood flow in healthy volunteers following acute oral and intravenous administration. In one study after one week of treatment with orally administered indomethacin, this effect on basal cerebral blood flow had disappeared. The clinical significance of this effect has not been established.

Pharmacokinetics

Bioavailability : Oral 100%, Rectal 80%

Metabolism: Hepatic-glucoronidation, demethylation,

deacylation

Elimination half-life: 2 hrs (terminal 4 to 11 hrs due to

enterohepatic recycling)

Excretion : Unknown, most probably as well biliar and

in urine

Following single oral doses of Capsules indomethacin 25 mg or 50 mg, indomethacin is readily absorbed, attaining peak plasma concentrations of about 1 and 2 mcg/mL, respectively, at about 2 hours. Orally administered Capsules of indomethacin are virtually 100% bioavailable, with 90% of the dose absorbed within 4 hours.

Indomethacin is eliminated via renal excretion, metabolism, and biliary excretion. Indomethacin undergoes appreciable enterohepatic circulation. The mean half-life of indomethacin is estimated to be about 4.5 hours.

Indomethacin exists in the plasma as the parent drug and its desmethyl, desbenzoyl, and desmethyldesbenzoyl metabolites, all in the unconjugated form. About 60 percent of an oral dosage is recovered in urine as drug and metabolites (26 percent as indomethacin and its glucuronide), and 33 percent is recovered in feces (1.5 percent as indomethacin).

About 99% of indomethacin is bound to protein in plasma over the expected range of therapeutic plasma concentrations. Indomethacin has been found to cross the blood-brain barrier and the placenta.

Indications

Indomethacin capsules have been found effective in active stages of the following:

- Moderate to severe rheumatoid arthritis including acute flares of chronic disease.
- Moderate to severe ankylosing spondylitis.
- Moderate to severe osteoarthritis.
- Acute painful shoulder (bursitis and/or tendinitis).
- Acute gouty arthritis.
- Juvenile arthritis
- Psoriatic arthritis
- Reiter's disease
- · Paget's disease of bone
- Bartter's disease
- Pseudogout
- Dysmenorrhea (menstrual cramps)
- Pericarditis
- Nephrogenic diabetes insipidus (prostaglandin inhibits vasopressin's action in the kidney)
- Fever and pain associated with malignant diseases (tumors, bony metastasis, lymphogranulomatosis)

Indomethacin has also been used clinically to delay premature labor, reduce amniotic fluid in polyhydramnios, and to treat **patent ductus** arteriosus.

Indomethacin is a potent drug with many serious side effects and should not be considered an analgesic for minor aches and pains or fever. The drug is more potent than Aspirin, but the usually tolerated doses of

Indomethacin do not allow a superior efficiency compared to aspirin. In mild to moderate pain a usual oral dose of Indomethacin proved as efficient as 600 mg aspirin.

Indomethacin may enable the reduction of steroid dosage in patients receiving steroids for the more severe forms of rheumatoid arthritis. In such instances the steroid dosage should be reduced slowly and the patients followed very closely for any adverse effects

Contraindications

Indomethacin should not be used in:

- Patients who are hypersensitive to this product.
- Patients in whom acute asthmatic attacks, urticaria, or rhinitis are precipitated by aspirin or other nonsteroidal anti-inflammatory agents.
- Indomethacin suppositories are contraindicated in patients with a history of proctitis or recent rectal bleeding.
- · Acutely existing ulcer ventricular and/or duodenal, or history of ulcer
- Children under 2 years of age
- Severe preexisting renal and liver damage
- Caution: preexisting bone marrow damage (frequent blood cell counts indicated)
- Caution: Bleeding tendencies of unknown origin (Indomethacin inhibits platelet aggregation)
- Caution: Parkinson, epilepsy, psychic disorders (Indomethacin may worsen these conditions)

Adverse Reactions

Since indomethacin inhibits both COX-1 and COX-2, it inhibits the production of prostaglandins in the stomach and intestines, which maintain the mucous lining of the gastrointestinal tract. Indomethacin, therefore, like other nonselective COX inhibitors, can cause ulcers. The ulcers can result in serious bleeding and/or perforation requirering hospitilization of the patient.

Some even die from these complications. To reduce the possibility of peptic ulcers, indomethacin should be prescribed at the lowest dosage needed to achieve a therapeutic effect, usually between 50–200 mg/day. It should always be taken after a meal. Nearly all patients benefit from an ulcer protective drug (e.g. highly dosed antacids, ranitidine 150mg at bedtime, or omeprazol 20 mg at bedtime). Other common seen gastrointestinal complaints as dyspepsia, heartburn and mild diarrhea are harmless in nature and rarely require discontinuation of Indomethacin.

Many NSAIDs, but particularly indomethacin, cause lithium retention by reducing its excretion by the kidneys. Thus indomethacin users have an elevated risk of lithium toxicity. For patients taking lithium supplements (e.g. for treatment of depression or bipolar disorder), less toxic NSAIDs such as sulindac or aspirin, are preferred.

Indomethacin also reduces plasma renin activity and aldosterone levels, and increases sodium and potassium retention. It also enhances the effects of vasopressin. Together these may lead to:

Edema (swelling due to fluid retention)

Hyperkalemia (high potassium levels)

Hypernatremia (high sodium levels)

Hypertension (high blood pressure)

The drug may also cause elevations of serum creatinine and more serious renal damage such as acute renal failure, chronic nephritis and nephrotic syndrome. These conditions also often begin with edema and hyperkalema.

Additionally, Indomethacin quite often causes headache (10 to 20%), sometimes with vertigo and dizziness, hearing loss, tinnitus, blurred vision with or without retinal damage and worsens Parkinson's Disease, epilepsy, and psychic disorders. Cases of life-threatening shock (including angioedema, sweating, severe hypotension and tachycardia as well as acute bronchospasm), severe or lethal hepatitis and severe bone marrow damage

have all been seen. Skin reactions and photosensitivity are also possible side effects.

Due to its strong antipyretic activity Indomethacin may obscure the clinical course of serious infections.

The frequency and severity of side effects and the availability of better tolerated alternatives make Indomethacin today a drug of second choice. Its use in acute gout attacks and in dysmennorhea is well established because in these indications the duration of treatment is limited to a few days only, therefore serious side effects are not likely to occur.

Cardiovascular

: Thrombophlebitis

Hematologic

: Although there have been several reports of

leukemia, the supporting information is weak

Genitourinary

: Urinary frequency.

A rare occurrence of fulminant necrotizing fasciitis, particularly in association with Group A hemolytic streptococcus, has been described in persons treated with non-steroidal anti-inflammatory agents, including indomethacin, sometimes with fatal outcome

Drug Interactions

ndomethacin potentiates cumarin anticoagulant activity, activity of the oral antidiabetics, corticosteroids, nifedipine, and verapamil. Concomitant administration with other NSAID and aspirin increases the risk of ulceration. Indomethacin reduces the renal clearance of lithium preparation; inhibits the vasodilating effect of nitroglycerin; increases toxicity of the aminoglycoside antibiotics.

Special Warnings and Precautions

Pregnancy category

C: 1st and 2nd. trimenion, D: 3rd. trimenion (possible damage for fetus exists)

Legal status

Schedule "H" Drug

Indomethacin should be used with care in patients with renal and liver diseases; ulcer disease in remission (it is obligatory to add H2-blocker or other gastroprotective medicines). Systemic application of Indomethacin may cause changes in several laboratory tests, including: elevation of the glucose, bilirubin, transaminases, creatine, and urea serum level; prolongs the bleeding time; reduced the creatine clearance; and reduces osmolality of the urine. Application of the tablets and suppositories in drivers and machine operating persons is not recommendable, as the medicine may provoke side reactions (somnolence, color sensitivity disorders, and diplopia), disturbing the concentration capabilities.

Animal Toxicity and Human Overdose

LD50: Oral LD50 of indomethacin in mice and rats (based on 14 day mortality response) is 50 and 12 mg/kg, respectively.

Indomethacin has a high acute toxicity both for animals and for humans. Exact human data does not exist, but some fatal human cases, particular in children and adolescents, have been seen.

Generally, overdose in humans causes drowsiness, dizziness, severe headache, mental confusion, paraesthesia, numbness of limbs, nausea and vomiting. Severe gastrointestinal bleeding is also possible. Cerebral edema, and cardiac arrest with fatal outcome have been seen in children. The treatment is symptomatic and largely the same as with diclofenac. However, the possibility of severe GI tract symptoms should be particularly noted. The risk of overdose after exaggerated local treatment with gel or spray is very limited.

Routes of Administration

Oral, rectal, sometimes intra-muscular and local (spray and gel).

Dosage Forms

- Tablets or Capsules 25 and 50mg
- Suppositories 50 and 100mg
- SR Capsules 75mg

- Syrup/Suspension (25mg/5ml)
- Injectable concentrate 50mg for i.m. injection
- Spray or Gel
- Indomethacin sodium trihydrate lyophilized powder for injection (Indomethacin I.V.), equivalent to indomethacin 1 mg, single-dose vials.

Storage

Store in well-closed, light-resistant containers Capsules should be kept at room temperature 15-30°C (59-86°F). Oral suspension and suppositories should be kept below 30°C (86°F). The oral suspension should not be frozen.

ANALYTICAL METHODS OF INDOMETHACIN

A titrimetric analysis method for the assay of Indomethacin by titrating with 0.1M sodium hydroxide solution using phenolphthalein solution as indicator is given in BP^{52} and IP^{53} .

Grippa et al.⁵⁴ demonstrated HPLC method for simultaneous determination of hydrocortisone dexamethasone, indomethacin, phenylbutazone and oxyphenbutazone in equine serum using probenecid as internal standard and C-18 column equilibrated with 51:49 acetonitrile: Water, 0.1% trifluroacetic acid at 1 ml/min. The elute was monitored at 254 nm.

Novakova et al.⁵⁵ described a fast simple and fully automateted HPLC method for determination of indomethacin and its two-degradation product in pharmaceutical preparation. HPLC was performed using Zorbax SB-CN (150 mm x 4.6 mm, 5:m) analytical columns and Zorbax phenyl analytical column (75 mm x 4.66 mm, 3.5:m) and mobile phase composed of acetonitrile and 0.2% Phosphoric acid (50: 5, V/V) at flow rate 0.6 ml/min for former column and 1.2 ml/min for later. Both methods used detection wavelength 237 nm and ketoprofen or flurbiprofen as internal standard for quantitation.

Bayne et al.⁵⁶ reported HPCL method for estimation of indomethacin in Biological fluids. They used Hypersil ODS column with 76% methanol in 0.025

Kwong et al.⁵⁷ described HPLC method for determination of indomethacin in pharmaceutical dosage form using ultrasphere ODS column and methanol-water- acetonitrile-acetic acid (55: 35: 10: 1) as mobile phase with UV detection at 254 nm

Terweij-Groen et al. 58 described HPLC method for determination of indomethacin in biological fluid using Zorbax ODS column and optimum mobile phase with UV detection at 235 nm.

Cotton⁵⁹ described HPLC method for determination of indomethacin in pharmaceutical dosage using silica, 10 um, column and gradient A: 8% acetic acid in heptane B: 8% acetic acid and 20% ethanol in heptane as mobile phase with UV-detection at 254 nm.

Brien⁶⁰ described HPLC method for determination of indomethacin in pharmaceutical dosage using C18 uBondapak column and 27% acetonitrile in 1% aqueous formic acid as mobile phase with UV determination at 254 nm.

Roman⁶¹ described HPLC method for determination of indomethacin in pharmaceutical dosage using C18 uBondapak column and 60% methanol in 2.5% phosphoric acid as mobile phase with ultraviolet detection at 240 nm.

 ${
m Wu}^{62}$ described HPLC method for determination of indomethacin in bulk drug using C18 uBondapak column and gradient A: 250 ml acetonitrile in 750 phosphate buffer (pH 7, 0.02 M) B: 700 ml acetonitrile in 300 as mobile phase with UV detection at 254 nm.

Holt et al.⁶³ described fluorescence analysis of Indomethacin hydrolysis product 2-methyl-5-methoxy indole acetic acid, which fluoresces at 385 nm after excitation at 300 nm in 0.1 N NaOH.

Hvidberg et al.⁶⁴ described fluorescence analysis of indomethacin at 387 nm after excitation at 312 nm in pH 11.6 buffer.

A spectrophotometric estimation of indomethacin in capsules and suppositories after dissolving in 1: 9 water and methanol then suitable diluted

with equal volume of methanol and phosphate buffer pH 7.2 is given in BP⁶⁵. The absorbance was measured at 320 nm for capsules & 318 nm for suppositories.

A similar spectrophotometric estimation methods of indomethacin in capsules and suppositories is given in IP⁶⁶ where dosage form is dissolve in 1: 9 water and methanol (for capsules) or in methanol (for suppositories) then suitably diluted with equal volume of methanol and phosphate buffer pH 7.2. The absorbance was measured at 320 nm.

Kazemifard and Holleck⁶⁷ described polarographic analysis method specific for nonhydrolysed indomethacin and suitable for analysis of capsules, suppositories and suspension with precision of ± 1.2 %, ± 0.7 %, and ± 1.2 % for respective formulation

Plazonnete and Vandenheuvel⁶⁸ reported GC-M.S characterization of indomethacin using trimethylsilyl esters separated on SE-52 liquid phase they also reported precision and accuracy using an electron capture detector, which for plasma was 92±19% (5 ng/ml) and 96±1.5% (1000 ng/ml). For aqueous humors, the values were 97±5.6% and 99±2.2% respectively.

Ferry et al.⁶⁹ have determined indomethacin as its ethyl esters on a 1mX2.5mm column packed with 100-120 Mesh chromosorb W (AW-DMCS) coated with 2% (w/w) OV-1 under optimum conditions utilizing an electron capture detector, a recovery from spiked serum of 96± 3% was attained.

Jambhekar et al. 70 utilized HPLC method for estimation of indomethacin in rabbit blood plasma. Calibration curve was obtained with correlation coefficient (r^2) 0.997 and recovery accuracy of 85.4 \pm 4.9% phenylbutazone was used as internal standard and acetonitrile: water in ratio of 65: 35 as mobile phase.

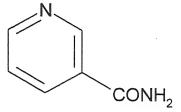
Taylor et al.⁷¹ reported the use of high-performance liquid chromatography-electrospray-tandem mass spectrometry (HPLC-ESI-MS/MS) for the quantification of indomethacin (IND) in plasma with microscale sample preparation. Plasma samples (100 [micro]L) and mefanamic acid (internal standard [IS]), buffered to pH 3.5, were prepared using solid phase extraction

and chromatographed using a C8 column. The mobile phase composition was 80% methanol to 20% ammonium acetate buffer (40 mM, pH 5.1). A flow rate of 300 [micro]L per minute was used with a 1-to-12 postcolumn split into the mass spectrometer. Selected reaction monitoring with mass transitions m/z 357.9 -> 139.0 and m/z 242 -> 209.0 were used for IND and IS, respectively. The chromatographic analysis time was 4 minutes. The assay was linear from 5 [micro]g/L to 2000 [micro]g/L with interday imprecision (n = 5) over the analytic range (5%). At four concentrations (10 [micro]g/L, 25 [micro]g/L, 1500 [micro]g/L), assay imprecision was 9% (total coefficient of variation [CV]) and accuracy ranged between 96.5% and 102.8% (n = 16). The absolute recovery of IND and IS was 74% (n = 8) and 95% (n = 24), respectively. This method was developed and validated in less than 10 working days, had a lower limit of quantification than reported HPLC-ultraviolet (UV) methods, and uses small sample volumes. These factors illustrate the power of HPLC-ESI-MS/MS for drug analysis. Furthermore, the ability of this method to measure IND over a wide concentration range makes it suitable for therapeutic drug monitoring and pharmacokinetic studies.

NICOTINAMIDE72-73

(Niacinamide)

Nicotinamide is pyridine-3-carboxamide.



Chemical Formula: C₆H₆N₂O

Mol. Wt.: 122.13

Action and use: Component of vitamin B.

Dose: Orally, prophylactic, 15 to 30 mg daily; therapeutic, 50 to 250 mg daily.

By intravenous injection, 50 to 250 mg daily.

Description: Colourless crystals or white, crystalline powder; odour, faint and

characteristic.

Solubility: Freely soluble in water and in ethanol (95%); slightly soluble in

chloroform and in ether.

Storage: Store in well-closed containers.

Standards

Nicotinamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_6N_2O$, calculated with reference to the dried substance.

Identification

A. Melting point (2.2.14): 128°C to 131°C.

B. Examine by infrared absorption spectrophotometry, comparing with the spectrum obtained with nicotinamide CRS.

pH: Between 6.0 and 7.5, determined in a 5.0% w/v solution,.

Clarity and colour of solution: A 5.0% w/v solution in carbon dioxide-free water is clear.and not more intensely coloured than reference solution BYS7,

Chloride: 1.0 g complies with the limit test for chlorides, (250 ppm).

Sulphate: 1.2 g complies with the limit test for sulphates, (125 ppm).

Loss on drying: Not more than 0.5%, determined on 1 g by drying over phosphorus pentoxide at a pressure of 1.5 to 2.7 kPa for 18 hours,

RESORCINOL73

Chemical Formula: C₆H₆O₂

Mol. Wt.: 110.1

Action and use: Keratolytic.

Description: A colourless or slightly pinkish-grey, crystalline powder or crystals, turning red on exposure to light and air, very soluble in water and in alcohol, freely soluble in ether.

Storage: Store in a well-closed container, protected from light.

Standards

Resorcinol contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of benzene-1,3-diol, calculated with reference to the dried substance.

Identification

A. Melting point: 109°C to 112°C.

B. Dissolve 0.1 g in 1 ml of water R, add 1 ml of strong sodium hydroxide solution R and 0.1 ml of chloroform R, heat and allow to cool. An intense, deep-red colour develops which becomes pale yellow on the addition of a slight excess of hydrochloric acid R.

Clarity and colour of solution: A 10.0% w/v solution (solution A) is clear and not more intensely coloured than reference solution B₅ or R₅ and remains so when heated in a water-bath for 5 min.

Acidity or alkalinity: To 10 ml of solution A add 0.05 ml of bromophenol blue solution R2. Not more than 0.05 ml of 0.1M hydrochloric acid or 0.1M sodium hydroxide is required to change the colour of the indicator.

Loss on drying: Not more than 1.0 per cent, determined on 1.00 g of powdered substance by drying in a desiccator for 4 h.

UREA⁷²⁻⁷³

Urea is the diamide of carbonic acid.

Chemical Formula: CH₄N₂O

Mol. Wt.: 60.06

Action and use: Keratolytic.

Dose: 5 to 15 g.

Description: White, crystalline powder or transparent crystals; odourless or almost odourless, but may gradually develop a slight odour of ammonia upon long standing; slightly hygroscopic.

Solubility: Freely soluble in water and in boiling ethanol (95%); soluble in ethanol (95%); practically insoluble in chloroform, in dichloromethane and in ether.

Storage: Store in well-closed containers.

Standards

Urea contains not less than 99.0 per cent and not more than 101.0 per cent of CH_4N_2O , calculated with reference to the dried substance.

Identification

A. Melting point: 132°C to 135°C.

B. Examine by infrared absorption spectrophotometry, comparing with the spectrum obtained with urea CRS. Examine the substances prepared as discs.

Alkalinity: To 10 ml of a 5.0% w/v solution (solution A) add 0.1 ml of methyl red solution and 0.4 ml of 0.01M hydrochloric acid; the resulting solution is red to orange.

Clarity and colour of solution: Solution A is clear, and colourless.

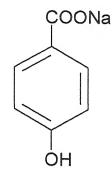
Loss on drying: Not more than 1.0%, determined on 1 g by drying in an oven at 105° for 1 hour.

SODIUM PARA- HYDROXY BENZOATE

Chemical Formula: C7H5NaO3

Mol. Wt.: 160.12

Action and use: Pharmaceutical aid



Description: White, crystalline or granular powder or flakes; odourless or with a faint odour; hygroscopic.

Solubility: Freely soluble in water; sparingly soluble in ethanol (95%).

Storage: Store in well-closed containers.

Standards

Sodium p-hydroxy benzoate contains not less than 99.0 per cent and not more than 100.5 per cent of $C_7H_5NaO_3$, calculated with reference to the dried substance.

Identification:

A: To a 10% w/v solution add ferric chloride test-solution; a buff coloured precipitate is formed. Add dilute hydrochloric acid; a white crystalline precipitate is produced.

B: Gives the reactions of sodium salts and reactions B and C of benzoates.

Acidity or alkalinity: To 20 ml of a 5% w/v solution in carbon dioxide-free water add 0.2 ml of phenolphthalein solution. Not more than 0.2 ml of 0.1M hydrochloric acid or 0.2 ml of 0.1M sodium hydroxide is required to change the colour of the solution.

Clarity and colour of solution: A 10.0% w/v solution in carbon dioxide-free water is clear, and not more intensely coloured than reference solution YS6,

Loss on drying: Not more than 2.0%, determined on 1 g by drying in an oven at 105°.

COONa

SODIUM BENZOATE72-73

Chemical Formula: C7H5NaO2

Mol. Wt.: 144.11

Action and use: Pharmaceutical aid (preservative).

Description: White, crystalline or granular powder or flakes; odourless or with

a faint odour, hygroscopic.

Solubility: Freely soluble in water; sparingly soluble in ethanol (95%).

Storage: Store in well-closed containers.

Standards

Sodium Benzoate contains not less than 99.0 per cent and not more than 100.5 per cent of C₇H₅NaO₂, calculated with reference to the dried substance.

Identification:

A: To a 10% w/v solution add ferric chloride test-solution; a buff coloured precipitate is formed. Add dilute hydrochloric acid; a white crystalline precipitate is produced.

B: Gives the reactions of sodium salts and reactions B and C of benzoates.

Acidity or alkalinity: To 20 ml of a 5% w/v solution in carbon dioxide-free water add 0.2 ml of phenolphthalein solution. Not more than 0.2 ml of 0.1M hydrochloric acid or 0.2 ml of 0.1M sodium hydroxide is required to change the colour of the solution.

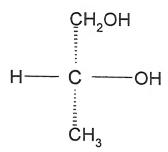
Clarity and colour of solution: A 10.0% w/v solution in carbon dioxide-free water is clear, and not more intensely coloured than reference solution YS6,

Loss on drying: Not more than 2.0%, determined on 1 g by drying in an oven at 105°

PROPYLENE GLYCOL72-74

1, 2-Propanediol

Propylene Glycol is (RS)-propane-1,2-diol.



Chemical Formula: C₃H₈O₂

Mol. Wt.: 75.09

Action and use: Pharmaceutical aid (humectant; solvent).

Description: Clear, colourless, viscous liquid; practically odourless; hygroscopic.

Solubility: Miscible with water, with ethanol (95%), with acetone and with chloroform.

Storage: Store in tightly-closed containers.

Clarity and colour: The substance being examined is clear, and colourless

Boiling range: Between 184° and 189°.

Relative density: Between 1.035 and 1.040, Refractive index: Between 1.431 and 1.433.

Incompatibilities: PG is incompatible with oxidizing reagents such as potassium permanganate.

Safety: Propylene glycol is used in variety of pharmaceutical formulations and is generally regarded as a non-toxic material. In topical preparations propylene glycol is regarded as minimally irritant although it is more irritant than glycerin. Parenteral administration may cause pain or irritation when used in high concentration. It is estimated to be one third as intoxicating as ethanol with administration of large volumes.

Application: PG has become widely used as a solvent, extractant and preservative in variety of parenteral and non-parenteral pharmaceutical formulations.

CH₂OH

СНОН

CH₂OH

GLYCERIN^{72,73,75}

Glycerol

Glycerin is propane-1,2,3-triol.

Chemical Formula: C₃H₈O₃

Mol. Wt.: 92.09

Action and use: Lubricant; laxative; pharmaceutical aid (humectant).

Description: Clear, colourless or almost colourless, syrupy liquid; odourless;

very hygroscopic.

Solubility: Miscible with water and with ethanol (95%); slightly soluble in

acetone; practically insoluble in ether and in fixed oils and volatile oils.

Storage: Store in tightly-closed containers.

Standards

Glycerin contains not less than 98.0 per cent and not more than 101.0 per cent of C₃H₈O₃, calculated with reference to the anhydrous substance.

Refractive index: between 1.470 and 1.475, determined at 20°.

Clarity and colour of solution: Solution A is clear, Dilute 10 ml of solution A to 25 ml with water. The solution is colourless.

Incompatibilities: Glycerin may explode if mixed with strong oxidizing agents, such as chromium trioxide, potassium chlorate or potassium permanganate, Black discoloration of glycerin occurs, in the presence of light on contact zinc oxide or basic bismuth nitrate.

Glycerin forms a boric acid complex, which is a stronger acid.

Safety: Glycerin is used in a wide variety of pharmaceutical formulation including oral, ophthalmic, parenteral and topical preparation. Adverse effects are mainly due to the dehydrating proportion of glycerin. Oral doses are demulcent and mildly laxative in action.

Application: Glycerin is used in a wide variety of pharmaceutical formulation including oral, otic, ophthalmic, topical and parenteral preparation. It is also used in cosmetics and as food additive. In topical formulation and cosmetics, glycerin is used primarily for its humectant and emollient properties. In parenteral formulations glycerin is used as a solvent while in oral solution it is used as sweetening agent, antimicrobial preservative and viscosity increasing agent. It is also used as a plasticizer of gelatin in production of soft gelatin capsules and gelatin suppositories.

ETHANOL72,73,76

Absolute Alcohol; Dehydrated Alcohol (CH₃CH₂OH)

Chemical Formula: C₂H₆O

Mol. Wt.: 46.07

Action and use: Pharmaceutical aid (solvent).

Description: Clear, colourless, mobile and volatile liquid; odour, characteristic and spirituous; hygroscopic. Readily volatilises even at low temperature; boils at 78°; flammable, burning with a blue, smokeless flame.

Solubility: Miscible with water, with chloroform, with ether and with glycerin.

Storage: Store in tightly-closed containers in a cool place, away from fire and protected from moisture

Standards

Ethanol contains not less than 99.0 per cent w/w and not more than 100.0 per cent w/w, corresponding to not less than 99.4 per cent v/v and not more than 100.0 per cent v/v, at 15.56° , of C_2H_6O .

Relative density: Between 0.7871 and 0.7902, at 25°.

Acidity or alkalinity: To 20 ml add 0.25 ml of phenolphthalein solution; the solution remains colourless and requires not more than 0.2 ml of 0.1M sodium hydroxide to produce a pink colour.

Clarity of solution: Dilute 5.0 ml to 100.0 ml with water. The solution is clear, Cool to 10° for 30 minutes; the solution remains clear.

Appearance: It is clear and colourless—when compared with water R. Dilute 1.0 ml to 20 ml with water R. After standing for 5 min, the dilution remains clear when compared with water R

Acidity or alkalinity: To 20 ml add 20 ml of carbon dioxide-free water R and 0.1 ml of phenolphthalein solution R. The solution is colourless. Add 1.0 ml of 0.01M sodium hydroxide. The solution is pink (30 ppm, expressed as acetic acid).

Absorbance: Examined between 235 nm and 340 nm, the absorbance measured in a 5 cm cell using water R as the compensation liquid is not greater than 0.40 at 240 nm, 0.30 between 250 nm and 260 nm, and 0.10 between 270 nm and 340 nm. The absorption curve is smooth.

Residue on evaporation: Evaporate 100 ml to dryness on a water- bath and dry at 100°C to 105°C for 1 h. The residue weighs not more than 2.5 mg (25 ppm m/V).

Incompatibilities: In acidic condition, ethanol solution may react vigorously with oxidizing material. Mixtures with alkali may darken in color due to a reaction with residual amounts of aldehyde. Ethanol solutions are also incompatible with aluminum containers.

Safety: Ethanol is widely used in variety of pharmaceutical formulation and cosmetics ethanol is also consumed in alcoholic beverages. Ethanol is a central nervous system depressant and ingestion of low to moderate quantities and lead to symptoms of intoxication. Parenteral products, containing upto 50% of alcohol have been formulated. However such concentration can produce pain on intramuscular injection and lower concentrations such as 5-10% v/v are preferred.

Application: Ethanol is primarily used as a solvent, it is employed in solution as an antimicrobial preservative. Topical ethanol solution are also used as penetration enhances and as disinfectants.

POLYETHYLENE GLYCOL (PEG)^{72,77}

Macrogol, carbowaxes.

Structural formula:

PEG 200: HO CH₂ (CH₂OCH₂)_{4.2} CH₂ OH

PEG 400: HO CH₂ (CH₂OCH₂)_{8,7} CH₂ OH

Polyethylene glycol is α - Hydro - ω - hydroxy- Poly (oxy-1, 2-ethanediyl).

Chemical Formula: HO CH₂ (CH₂OCH₂)_m CH₂OH

m = average number of oxyethylene groups.

Molecular weight: PEG 200 190-210

PEG 400 380-420

Description: Polyethylene glycol are clear, viscous liquids at room temperature. They do not hydrolyse or deteriorate under typical conditions. As the molecular weight increases their water solubility, vapor pressure, hygroscoicity ad solubility in organic solvents decreases and freezing or melting range, specific gravity, flash point and viscosity increases.

Solubility: Soluble in water and miscible in all proportions with other PEGs. Liquid PEGs are soluble in acetone, alcohols, benzene, glycerin and glycols.

Other properties:

Density: 1.11-1.14g/cm³ at 25° C for liquid PEGs

Freezing point: <-65°C for PEG 200

80°C for PEG 400

Refractive index: 1.459 for PEG 200

1.465 for PEG 400

Viscosity: 39.9mm²/s at 25^oC for PEG 200

90.0mm²/s at 25^oC for PEG 400

Stability and storage conditions: Polythylene glycols are chemically stable in air and in solution. PEG do not support microbial growth nor they become rancid. Polyethylene glycols should be stored in well closed containers in cool, dry place. Stainless steel, aluminium, glass or lined steel containers are preferred for storage of liquid grades.

Incompatibilities: Liquid and solid polyethylene glycol grades may be incorporated with some colour. The antibacterial activity of certain antibiotics is reduced in PEG. The preservative efficacy of parabens may also be impaired. Migration of PEG can occur from tablet film coatings, leading to interaction with core components.

Safety: PEGs are widely used in variety of pharmaceutical formulation. Generally they are regarded as non-toxic and non-irritant materials. Oral administration of large quantities of polyethylene glycols can have laxative effect. Liquid PEG may be absorbed when taken orally, but the higher molecular weight PEGs are not significantly absorbed from gastrointestinal tract.

Application: Polyethylene glycols are widely used in a variety of pharmaceutical formulations including parenteral, topical, ophthalmic, oral and rectal preparations. PEGs are used as ointment bases. Mixtures of PEGs can be used as suppository bases. They are also used as suspending agents or to adjust viscosity and consistency of other suspending vehicles. Liquid PEGs are used as water-miscible solvents for the contents of soft gelatin capsules. In concentrations up to approximately 30% v/v, PEG 300 and PEG 400 have been used as the vehicle for parenteral dosage forms.

POLYSORBATE 20⁷²⁻⁷³

Polyoxyethylene 20 Sorbitan Monolaurate

$$\begin{array}{c|c} & (\mathsf{OCH_2CH_2})_{\mathsf{c}}\mathsf{OH} \\ & \mathsf{HO}(\mathsf{CH_2CH_2O})_{\mathsf{a}} & \mathsf{HC} \\ & \mathsf{CH_2}(\mathsf{OCH_2CH_2})_{\mathsf{d}}\mathsf{OCO}(\mathsf{CH_2})_{\mathsf{10}}\mathsf{CH_3} \\ & & (\mathsf{OCH_2CH_2})_{\mathsf{b}}\mathsf{OH} \end{array}$$

Polysorbate 20 is a mixture of partial lauric esters of D-glucitol and its anhydrides copolymerised with approximately 20 moles of ethylene oxide for each mole of D-glucitol and its anhydrides. The lauric acid used for the esterification may contain other fatty acids.

Action and use: Pharmaceutical aid (non-ionic surfactant).

Description: Clear or slightly opalescent, oily, yellowish or brownish yellow liquid; odour, faint and characteristic.

Solubility: Miscible with water, with ethanol, with ethyl acetate and with methanol; practically insoluble in fixed oils and in liquid paraffin.

Storage: Store in tightly-closed, light-resistant containers.

pH: Between 5.0 and 7.0, determined in a 5% w/v solution in carbon dioxide-free water

Acid value: Not more than 2.0, determined on 5 g

Hydroxyl value: Between 96 and 108, determined on 2 g by

lodine value: Not more than 5.0, determined by the iodine bromide method,

Saponification value: Between 40 and 50, using 15 ml of 0.5M ethanolic potassium hydroxide and diluting with 50 ml of water before carrying out the titration

POLYSORBATE 80⁷²⁻⁷³

Polyoxyethylene 20 Sorbitan Monooleate

$$\begin{array}{c} \text{HO(CH}_2\text{CH}_2\text{O})_a \\ \text{HC} \\ \text{CH}_2\text{(OCH}_2\text{CH}_2\text{)}_d\text{OCO(CH}_2\text{)}_{10}\text{CH}_3 \\ \text{COCH}_2\text{CH}_2\text{)}_b\text{OH} \\ \text{CH}_3\text{(CH}_2\text{)}_6\text{CH}_2 \\ \text{H} \end{array}$$

Polysorbate 80 is a mixture of partial oleic esters of D-glucitol and its anhydrides copolymerised with approximately 20 moles of ethylene oxide for each mole of D-glucitol and its anhydrides.

Action and use: Pharmaceutical aid (non-ionic surfactant).

Description: Clear or almost clear, oily, yellowish or brownish yellow liquid; odour, faint and characteristic.

Solubility: Miscible with water, with ethanol, with ethyl acetate and with methanol; practically insoluble in fixed oils and in liquid paraffin.

pH: Between 6.0 and 8.0, determined in a 5% w/v solution in carbon dioxide-free water

Acid value: Not more than 2.0, determined on 5 g

Hydroxyl value: Between 65 and 80, determined on 2 g by

lodine value: Between 18 and 24, determined by the iodine bromide method,

Saponification value: Between 45 and 55, using 15 ml of 0.5M ethanolic potassium hydroxide and diluting with 50 ml of water before carrying out the titration.

CH,OH

BENZYL ALCOHOL73

Chemical Formula: C7H8O

Mol. Wt.: 108.1

Action and use: Local anaesthetic; disinfectant.

Description:

A clear colourless, refringent, oily liquid, soluble in water, miscible with alcohol, with ether and with fatty and essential oils.

Standards

Benzyl alcohol contains not less than 97.0 per cent and not more than the equivalent of 100.5 per cent of phenylmethanol.

Identification

Add 0.1 ml to 5 ml of potassium permanganate solution R. Add 1 ml of dilute sulphuric acid R. The characteristic odour of benzaldehyde is produced.

Solubility: Shake 2 ml with 60 ml of water R. It dissolves completely giving a clear solution .

Acidity: To 10 ml add 10 ml of alcohol R and 1 ml of phenolphthalein solution R. Not more than 1 ml of 0.1M sodium hydroxide is required to change the colour of the indicator to pink.

Refractive index: 1.538 to 1.541.

Relative density: 1.043 to 1.049.

SODIUM METABISULPHITE⁷²⁻⁷³

Sodium Pyrosulphite; Sodium Disulphite

Chemical Formula: Na₂S₂O₅

Mol. Wt.: 108.1

Action and use: Pharmaceutical aid (antioxidant).

Description: Colourless, prismatic crystals or white or creamy white powder;

odour, sulphurous.

Storage: Store in tightly-closed, light-resistant containers in a dry place. On exposure to air and moisture it is slowly oxidised to sulphate with disintegration of the crystals.

Solubility: Freely soluble in water, slightly soluble in ethanol (95%).

Clarity and colour of solution: A 5.0 % w/v solution in carbon dioxide-free distilled water R is clear and colourless

pH: The pH of solution S is 3.5 to 5.0.

Acidity: A solution is acidic to phenol red solution.

GELUCIRE 44/14⁷⁸⁻⁸⁰

DEFINITION

Lauroyl Macrogolglycerides EP.

GELUCIRE 44/14 is composed of a well-defined mixture of mono-, di- and triglycerides and mono- and di- fatty acid esters of polyethylene glycol.

European Pharmacopoeia defines as "Mixture of monoesters, diesters and triesters of glycerol and monoesters and diesters of macrogols with a mean relative molecular mass between 300 and 1500".

Origin of the Raw Materials

GELUCIRE 44/14 is manufactured from raw materials of strictly vegetable and petrochemical origin.

They are obtained by partial alcoholysis of saturated oils mainly containing triglycerides of lauric acid, using macrogol with saturated fatty acids, or by mixing glycerol and macrogol with saturated fatty acids, or by mixing glycerol esters and condensates of ethylene oxide with the fatty acids of these hydrogenated oils.

Physical and Chemical Properties

Form : Waxy solid

Colour : Roughly white

Odour : Light

Melting Point : 42.5°C - 47.5°C

Boiling Point : >150°C Flash Point : >150°C

Self Igniting : Product is not self igniting

Solubility in / Miscibility with

Water : Soluble/Dispersible

Organic Solvents : Soluble in many organic solvents:

Ethanol 96°: Soluble

Chloroform, methylene chloride: Freely soluble

Mineral oils: Insoluble

Uses

Excipient for hard gelatin capsules, bioavailability enhancer.

- Increases solubility and bioavailability of poorly-soluble drugs.
- Protects drugs against oxidation and hydrolysis.
- Allows handling of low density products, toxic or low dose active drugs.
- Allows formulation of solid dosage forms with liquid actives.

Storage

Preserve in its original container and prevent exposure to air, light, heat and moisture. Melt between 50 and 80°C and homogenize before any use.

Toxicology

- Acute toxicity by oral route (rats): LD 50 > 2004 mg/kg
- 90-day oral subchronic toxicity (dogs): NOAEL > 2500 mg/kg/j
- Ames test: negative
- Micronucleus test: negative
- Mouse lymphoma assay: negative

DATA SHEET OF GELUCIRE 44/14

Appearance	waxy solid
Odour	faint
Colour (gardner scale)	< 5
Drop point (mettler)	42.5 to 47.5 °c
Acid value	< 2.00 mgkoh/g
Saponification value	79 to 93 mgkoh/g
lodine value	< 2 gl2/100g
Hydroxyl value	36 to 56 mgkoh/g
Peroxide value	< 6.0 meqo2/kg
Alkaline impurities	< 80 ppm naoh
Water content	< 0.50 %
Free glycerol content	< 3.0 %
Caprylic acid (C8)	< 15 %
Capric acid (C10)	< 12 %
Lauric acid (C12)	30 to 50 %
Myristic acid (C14)	5 to 25 %
Palmitic acid (C16)	4 to 25 %
Stearic acid (C18)	5 to 35 %
Total ashes content	< 0.10 %
Heavy metals	< 10 ppm
Ethylene oxide content	< 1 ppm
1.4 dioxane content	< 10 ppm

Source : Colorcon Asia Private Limited, Goa, India

HYDOXYPROPYL-β-CYCLODEXTRIN^{73,81-84}

Substitution : 0.65 Molecular Weight : 1396

Aqueous Solubility : >500 mg/ml

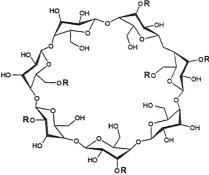
 $\mbox{Molecular Formula} \qquad : \quad (C_6 H_{10} O_5)_7 (C_3 H_6 O)_{4\text{-}5}$

Cavity Diameter : 6.0-6.5 Å

Cavity Height : 7.9 Å

Cavity Volume : 262 Å³

Number of Glucose Unit: 7



R = OCH₂CH(CH₃)OH

Hydroxypropyl- β -cyclodextrin (HP β -CD) is a modified β -CD obtained by treating a base-solubilized solution of β -CD with propylene oxide. This chemical modification significantly increases the solubility of HP β -CD over β -CD. In addition, HP β -CD is well tolerated and appears to be safe in clinical trials without observable renal toxicity as shown with β -CD. It is safely used as excipient in parenteral dosage forms and effects drug solubilization through the formation of dynamic (non-covalent) complex formation.

Cyclodextrins are bucket-shaped oligosaccharides produced from starch. As a result of their molecular structure and shape, they possess a unique ability to act as molecular containers by entrapping guest molecules in their internal cavity. The resulting inclusion complexes offer a number of potential advantages in pharmaceutical formulations.

Cyclodextrins are a general class of molecules composed of glucose units connected by α -1,4 glycosidic linkages to form a series of oligosaccaride rings.

In nature, the enzymatic digestion of starch by cyclodextrin glycosyltransferase (CGTase) produces a mixture of cyclodextrins comprised of 6, 7 and 8 glucose units (α , β and γ -cyclodextrin, respectively). Commercially, cyclodextrins are still produced from starch, but more specific enzymes are used to selectively produce consistently pure α , β or γ -cyclodextrin, as desired. These are:

- Thermally stable (< 200 °C)
- Very stable in alkaline solutions (pH < 14)
- Stable in acidic solutions (pH > 3)
- Biocompatible

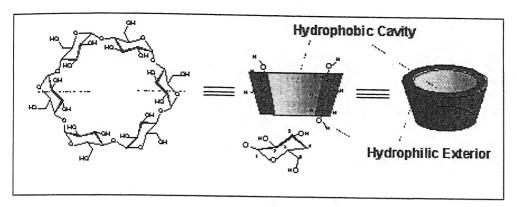
Cyclodextrin characteristics and key specifications

, , ,			r
Cyclodextrin Type	α	β	γ
Number of Glucose Units	6	7	8
Appearance	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Molecular Weight	973	1135	1297
Bulk Density, g/cm ³	0.4 - 0.7	0.4 - 0.7	0.4 - 0.7
Water Solubility (25 °C), g/100 mL	14.5	1.8	23.2
Content (dry basis)	>98%	>98%	>98%
Specific Rotation in Aqueous Solution [ঝ] _{D,20}	+147° to +152°	+160° to +164°	+174° to +180°
Water	<10%	<14%	<11%
Heavy Metals	<5 ppm	<5 ppm	<5 ppm
Residue on Ignition	<0.1%	<0.1%	<0.1%
Volatile Organics	<20 ppm	<5 ppm	<50 ppm
Micro-organisms	<1000/g	<1000/g	<1000/g

Cyclodextrin Complexes

The ability of a cyclodextrin to form an inclusion complex with a guest molecule is a function of two key factors. The first is steric and depends on the relative size of the cyclodextrin to the size of the guest molecule or certain key functional groups within the guest.

The most stable three dimensional structure of cyclodextrins is a toroid with the larger and smaller openings presenting hydroxyl groups to the external environment and mostly hydrophobic functionality lining the interior of the cavity. It is this unique configuration that gives cyclodextrins their interesting properties and creates the thermodynamic driving force needed to form host-guest complexes with apolar molecules and functional groups.



The conformation of the glucose units in the cyclodextrin places the hydrophilic hydroxyl groups at the top and bottom of the three dimensional ring and the hydrophobic glycosidic groups on the interior.

Applications

As a result of their unique ability to form inclusion complexes, cyclodextrins provide a number of benefits in pharmaceutical formulations. Many of these applications have been well-studied and a significant amount of information exists in the technical literature.

- Bioavailability Enhancement
- Active Stabilization
- Odor or Taste Masking
- Compatibility Improvement
- Material Handling Benefits
- Irritation Reduction

Quality, Safety and Regulatory Status

 α , β and γ -cyclodextrin are fully registered in all major chemical inventories world-wide (TSCA, EINICS/ELINCS, DSL, NDSL, ENCS (MITI), AICS, ECL). Being starch derivatives, cyclodextrins are generally regarded as essentially non-toxic materials. However, β -cyclodextrin can form insoluble complexes with cholesterol that disrupt the function of the kidneys so it should not be used in parenteral applications and its oral use should be limited to a daily maximum of 5 mg/kg. Both α and γ -cyclodextrin are suitable for oral

applications with acceptable daily intakes (ADIs) given as "not specified" by the JECFA (Joint FAO/WHO Expert Committee on Food Additives). γ -cyclodextrin can be also used parenterally. In the US both β and γ -cyclodextrin have GRAS status and can be used in food products. β -cyclodextrin is permitted in Europe as a food additive, the approval of γ -cyclodextrin under the Novel Food directive is pending.

Storage and Handling

Cyclodextrins have excellent stability when stored at ambient temperatures under dry conditions in their original containers. The retest interval for these products is 24 months. Continued storage beyond the retest interval does not imply that the material cannot be used. However, for proper quality assurance, the user should retest the product as appropriate for the intended application.

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CHAPTER

Preformulation Studies

Preformulation research relates to pharmaceutical and analytical investigations that both proceed and support formulation development efforts for all dosages forms. Taking into account early pharmacological and biopharmaceutical data preformulation studies yield keys information necessary to guide the formulator and analyst toward the development of an elegant, stable dosage form with good bioavailability .The general subject of preformulation research has been described in detail by several investigators1-⁵ and is in wide use throughout the pharmaceutical industries. These presentations have dealt mainly with studies design for solid dosage forms. Some specific applications have been made to certain areas of parenteral interest. 6-7.

Typical physicochemical properties of drug substances that either characterizes or may exert significant influence on the development of a parenteral formulation were performed.

3.1 PREFORMULATION STUDIES

3.1.1 Molecular structure and weight

These are the most characteristics of drug substances and are among the first items to be known. From the molecular structure the investigator can make initial judgments regarding potential properties and functional groups reactivities.

3.1.2 Color

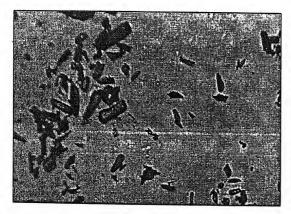
Color is generally a functional of a drug's inherent chemical structure relating to a certain level of unsaturation. Some compounds may appear to have color although structurally saturated. Such a phenomenon can often be due to the presence of minute traces of highly unsaturated, intensely colored impurities and/ or degradation products. These substances may be prone to increased color formation under stress conditions of heat, oxygen and light. A significant color change can become a limiting factor to the self-life of the parenteral product even before a significant change in chemical stability is noticed. The drug substances color should be recorded by subjective description, as well as by an objective means such as by comparison with standard color chips, or by spectrophometric analysis if the compound's color intensity in solution is proportional to concentration. The color of chlorzoxazone and indomethacin was observed visually and depicted in preformulation worksheet no. 1 and 2.

3.1.3 Odor

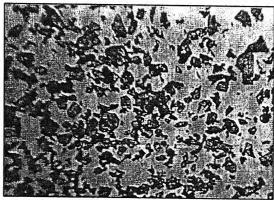
The odor of chlorzoxazone and indomethacin was examined by cautiously smelling the headspace of the drug container, which has been previously closed to allow volatiles to concentrate. The description of odor is recorded in preformulation worksheet no. 1 and 2.

3.1.4 Particle size, shape and crystallinity

Particle size and shape characteristics were determined by microscopic evaluation using an optical microscope with calibrated ocular micrometer. The morphological characteristics of the drugs were recorded by photomicrograph using Ezee capture camera. The average particle size of both the drugs are given in preformulation worksheet no. 1 and 2 and photomicrographs are shown below.



Photomicrograph of chlorzoxazone (100X)



Photomicrograph of indomethacin (100X)

3.1.5 Melting Point

The melting point of a substance is thermodynamically defined as the temperature at which the solid and liquid phases are in equilibrium as describe in the equation

Worksheet 1: Preformulation of Chlorzoxazone

1. 2. 3. 4. 5. 6. 7. 8. 9. 10.	Compound Name: Molecular Weight Molecular Formula Chemical Name Physical Appearance Odor Nature Particle Size M.P. Hygroscopicity Solubility at 25°C Solvent	Chlorzoxazone 169.58 C ₇ H ₄ CINO ₂ 5-chloro-3H-benzooxazol-2-one White to creamy white crystalline powder Odorless Crystalline powder 83.48±9.36 μm 194°C Non-hygroscopic
	 Water Methanol Ethanol Isopropanol Butanol Chloroform Ether DMSO DMF Acetone Phosphate Buffer pH-7.0 NaOH Solution Ammonia solution 	Practically insoluble Sparingly Soluble Freely Soluble Freely Soluble
12. 13. 14. 15.	Partition Coefficient (logP) pH Solubility Profile Hydrolysis Profile UV spectra	1.938±0.296 Fig. 3.1 and Table 3.2 Fig. 3.3 and Table 3.4
	 Spectra reference no. Solvent \(\frac{\lambda}{\max}\) max Concentration 	Fig. 3.5 Distilled water 280 nm 10 μg/ml
16.	FTIR spectraSpectra reference No.Characteristic Peaks	Fig. 3.7 Table 3.7
17.	Thermal AnalysisDSC curveEndothermic peakΔH	Fig. 3.9 194.399°C 132.707 J/g
18.	X-Ray Powder DiffractionDiffraction PatternCharacteristic Peaks	Fig. 3.11 Table 3.9
19.	Assay	99.68%

Worksheet 2: Preformulation of Indomethacin

1. 2. 3.	Compound Name: Molecular Weight Molecular Formula	Indomethacin 357.79 C ₁₉ H ₁₆ CINO ₄
4.	Chemical Name	1-(4-chlorobenzoyl)-5-methoxy-2-
5. 6.	Physical Appearance Odor	methylindol-3-ylacetic acid White to pale yellow Almost odorless
7.	Nature	Crystalline powder
8.	Particle Size	68.23±11.56 μm
9.	M.P.	161°C
10.	Hygroscopicity	Non-hygroscopic
11.	Solubility at 25°C	i i i i jg. deddpio
	Solvent	
	Water	Practically insoluble
	Methanol	Soluble
	Ethanol Isopropanol	soluble
	IsopropanolButanol	soluble Soluble
	Chloroform	Soluble
	• Ether	Soluble
	• DMSO	Soluble
	• DMF	Soluble
	Phosphate Buffer pH-7.0NaOH Solution	Sparingly Soluble
	- Naon Solution	Freely Soluble
12.	Partition Coefficient (logP)	3.172±0.258
13.	pH Solubility Profile	Fig. 3.2 and Table 3.3
14.	Hydrolysis Profile	Fig. 3.4 and Table 3.5
15.	UV spectra	
	 Spectra reference no. 	Fig. 3.6
	• Solvent	Distilled water
	 λ_{max} Concentration 	319.5 nm
16.		10 μg/ml
10.	FTIR spectra	5
	Spectra reference No.Characteristic Peaks	Fig. 3.8
17.	Thermal Analysis	Table 3.8
17.	•	Fi 0.40
	DSC curve Endethermain mark	Fig. 3.10
	Endothermic peak	161.533°C
18.	• ΔΗ X-Pay Paydor Diffraction	78.426 J/g
10.	X-Ray Powder Diffraction	F: 0.40
	Diffraction Pattern	Fig. 3.12
40	Characteristic Peaks	Table 3.10
19.	Assay	99.47%

A melting point determination is a good first indication of purity since the presence of relatively small amount of impurities can be detected by lowering as well as widening in the melting point range. Melting point for both drugs was determined using melting point apparatus and recorded in preformulation worksheet no. 1 and 2.

3.1.6 Hygroscopicity

Hygroscopicity studies were carried out over a range of humidity conditions. Samples of chlorzoxazone and indomethacin were accurately weighed into tarred containers and place at the various humidity conditions for period up to 2 weeks. Saturated solution of certain salts stored in sealed desiccators are used to establish well defined humidity conditions. These are shown in the table 3.1. Weight gained or loss was measured at predetermined intervals until equilibrium reached. An assessment was made regarding the relative weight gained as well as color and interpreted as non hygroscopic according to classification given by Callahan et al.8.

Table 3.1: Saturated salt solution for humidity control

Compound	% relative humidity	Temperature (°C)
Calcium Chloride (CaCl ₂ .6H ₂ O)	31	24.5
Potassium thiocynate (KSCN)	47	20.0
Sodium acetate (NaC ₂ H ₃ O ₂ .3H ₂ O)	76	20.0
Zinc sulphate (ZnSO ₄ .7H ₂ O)	90	20.0

3.1.7 Partition Coefficient

The partition coefficient P is a measure of lipophilicity of a compound and is expressed as by the equation

$$P = [C_{octanol}]/[C_{water}]$$

Log P value for chlorzoxazone and indomethacin were determined by shake flask method9. Accurately weighed amount (10mg) chlorzoxazone or indomethacin was placed in a separating funnel containing equal volumes of octanol and water (10 ml) then shaked vigorously for 30 min. The mixture was equilibrated for 24 hours with occasional shaking then the amount of drug present in aqueous medium was determined by UV visible spectrophotometer at 280.6 nm (for chlorzoxazone) and 319.5 nm (for indomethacin). Log P value was calculated and presented in preformulation worksheet no. 1 and 2.

3.1.8 Solubility

Preliminary solubility measurements were conducted to obtain the approximate solubility of chlorzoxazone and indomethacin in pure organic solvents. A known amount of chlorzoxazone or indomethacin (approximately 5 mg was placed into a small vial and 0.5 ml of solvent was added to the vial. The solution was agitated, more chlorzoxazone or indomethacin was added until either saturation was reached or about 50 mg of drug has been added to the 0.5 ml co-solvent. The results were interpreted as insoluble, slightly soluble, soluble or freely soluble and shown in preformulation worksheet preformulation worksheet no. 1 and 2.

3.1.9 pH - Solubility Profile

The compounds with either acidic or basic functionality will show difference in solubility characteristics with change in solution pH in accord with their ionization constants. These differences are often large and important in attaining the concentration of desired formulation.

The pH solubility of chlorzoxazone and indomethacin was determined at a pH ranging from 2.5-12 pH in phosphate buffer. An excess quantity of drug was placed in 10 ml screw capped culture tubes and mixed with buffer solution and stirred for 18 hrs at room temperature then the pH of the solution was measured and if needed readjusted. The samples were further stirred for 6 hrs and then the solution was centrifuged at 2000 rpm and the supernatant was filtered with Whattman filter paper no. 1 and analyzed by UV Spectrophotometer. Buffers in the pH range of 3.0-8.0 were prepared using 0.01 M citric acid and 0.02 M disodium hydrogen phosphate, in the pH range of 8.0-12.0 using 0.01 M glycine and 0.01 M sodium hydroxide and in the pH range below 3.0 using 0.01 M glycine and 0.01 M hydrochloric acid. pH higher then 8.0 was readjusted with 0.05 M NaOH during equilibration of the drugs. The pH shown in the table 3.2 and 3.3 are the pH of saturated solution of the drug. All the samples were prepared in triplicate. The solubility observed at various pH is given in table no. 3.2 and 3.3 and the profiles are shown in figure no 3.1 and 3.2.

Table 3.2: pH solubility data of chlorzoxazone

S.No.	pH of Solution	Solubility (mg/ml)	Enhancement Ratio
1.	2.55	0.324 ± 0.008	1.018
2.	3.09	0.325 ± 0.014	1.023
3.	4.14	0.329 ± 0.011	1.035
4.	5.96	$\textbf{0.330} \pm \textbf{0.014}$	1.036
5.	7.38	0.355 ± 0.010	1.115
6.	7.92	0.490 ± 0.009	1.541
7.	8.03	0.504 ± 0.028	1.585
8.	8.49	0.716 ± 0.024	2.248
9.	8.65	0.927 ± 0.030	2.911
10.	8.84	1.518 ± 0.033	4.768
11.	9.97	7.232 ± 0.688	22.720
12.	12.08	39.722 ± 1.500	124.787

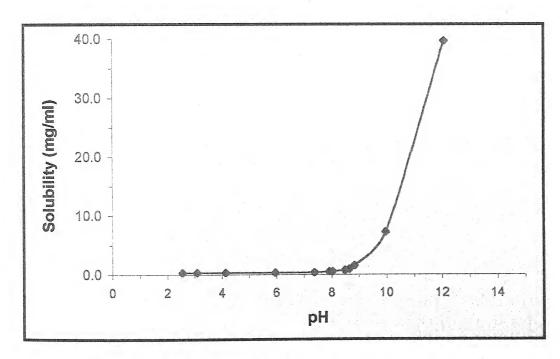


Fig. 3.1: pH solubility profile of chlorzoxazone

Table 3.3: pH solubility data of indomethacin

S.No.	pH of Solution	Solubility (mg/ml)	Enhancement Ratio
1.	2.45	0.003 ± 0.000	0.118
2.	3.02	0.003 ± 0.000	0.117
3.	4.04	0.005 ± 0.000	0.213
4.	4.98	0.014 ± 0.001	0.543
5.	6.01	0.320 ± 0.021	12.825
6.	6.56	0.884 ± 0.062	35.417
7.	7.07	3.124 ± 0.176	125.113
8.	7.62	3.542 ± 0.281	141.826
9.	7.96	3.640 ± 0.308	145.758

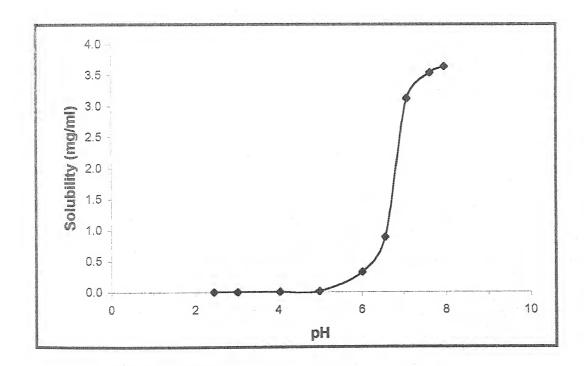


Fig. 3.2: pH solubility profile of indomethacin

3.1.10 Hydrolysis Profile

Hydrolysis profile of both drugs was determined at pH 3.0, 7.4 and 10.0 buffer at $25 \pm 2^{\circ}$ C, for 14 days. 300 mg of drug was added to screw capped 30 ml culture tubes and mixed with buffer solution of pH 3.0, 7.4 and 10.0 and stirred on vortex mixer then equilibrate for 4 hrs on rotary flask shaker, centrifuged and filtered. The drug content was determined by analyzing the solution by UV spectrophotometrically initially and after 1^{st} , 3^{rd} , 7^{th} , 10^{th} and 14^{th} day. These data are given in table no. 3.4 and 3.5. The hydrolysis rate constant was obtained from the slope of log % drug remaining vs. time plot (Fig. 3.3 and 3.4) and given in table 3.6.

Table 3.4: Hydrolysis profile of chlorzoxazone

Days —	Percentage drug remaining		
	pH 7.4	pH 3.0	pH 10.0
0	100.00 (4.6052)	100.00 (4.6052)	100.00 (4.6052)
1	99.94 (4.6046)	99.83 (4.6035)	99.64 (4.6016)
3	99.76 (4.6028)	99.67 (4.6019)	98.77 (4.5928)
7	99.41 (4.5993)	99.16 (4.5967)	97.24 (4.5772)
10	99.23 (4.5974)	98.88 (4.5939)	95.84 (4.5627)
14	99.09 (4.5960)	98.46 (4.5897)	93.89 (4.5421)

Values in parenthesis indicates natural log values

Table 3.5: Hydrolysis profile of indomethacin

Days —	Percentage drug remaining		
	pH 7.4	pH 3.0	pH 10.0
0	100 (4.6052)	100 (4.6052)	100 (4.6052)
1	99.96 (4.6048)	99.91 (4.6043)	99.64 (4.6016)
3	99.78 (4.6030)	99.72 (4.6024)	98.96 (4.5947)
7	99.48 (4.6000)	99.42 (4.5994)	98.11 (4.5861)
10	99.34 (4.5985)	99.25 (4.5976)	97.02 (4.5749)
14	99.15 (4.5966)	99.01 (4.5952)	95.79 (4.5622)

Values in parenthesis indicates natural log values



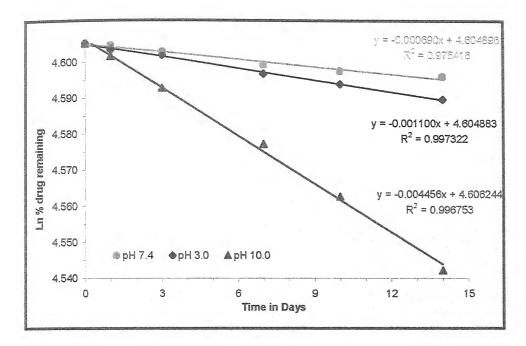


Fig. 3.3: Plot of hydrolysis profile of chlorzoxazone

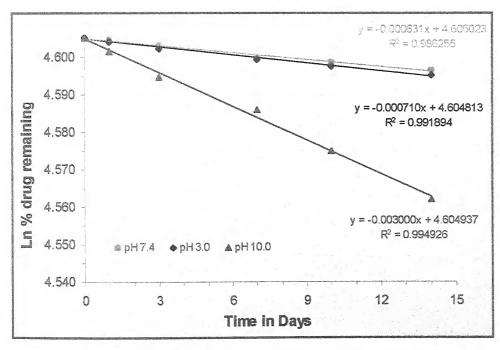
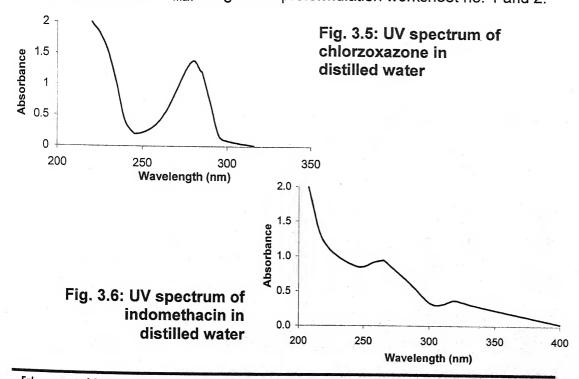


Fig. 3.4: Plot of hydrolysis profile of indomethacin

	Hydrolysis rate constant <i>K</i> (days ⁻¹) ×10 ⁴				
Drug		at different pH			
_	3.0	7.4	10.0		
Chlorzoxazone	11.00	6.90	44.56		
Indomethacin	7.10	6.31	30		

3.1.11 UV Spectral Studies

Molecules with structural unsaturation are able to absorb light within specific frequency range. The degree of unsaturation coupled with the presence of chromophores will influence the extent of absorption and whether UV (400-190 nm) or visible (800-400 nm) light will absorb. The UV spectra of compounds in solution are not highly specific; however they are very suitable for quantitative analytical work and serve as additional information for the compound identification. The UV spectrum of chlorzoxazone and indomethacin was determined by placing approximately a 10 $\mu g/ml$ solution of the compound in a 1 cm cell and recording the spectra versus the distilled water in the spectral range 200-400 nm using Simadzu-1701 (Japan) UV spectrophotometer. The spectra of both the drug are shown in figure 3.5 and 3.6 and the values of λ_{max} are given in preformulation worksheet no. 1 and 2.



3.1.12 Fourier Transform Infrared (FTIR) Spectral Studies

Fourier Transform Infrared Spectroscopy (FTIR) is another powerful technique for the physical characterization of pharmaceutical solids¹⁰. In the IR spectroscopy the vibrational modes of a molecule are used to deduce structural information. IR spectrum are best obtained on powder solids through the use of the diffuse reflectance method and interpreted through the conventional group frequency compilation¹¹. Three different spectral intervals are commonly identified: Far IR (100-400 cm⁻¹), mid IR (400-4000 cm⁻¹) and near IR (4000-14000 cm⁻¹) regions, although most applications have been made in the mid IR or near IR regions.

FTIR spectrum of chlorzoxazone and indomethacin were obtained by means of a FTIR spectrophotometer (FTIR-8400s Simadzu Japan). The samples were prepared by the potassium bromide disk method and measurements were attempted with the accumulation of 20 scans and a resolution of 4cm⁻¹ over the range of 400 to 4000 cm⁻¹. After the running a spectra significant peaks relating to major functional groups were identified, spectra of the subsequent sample of the same compound are compared with the original. The spectra are shown in figure 3.7 and 3.8 and characteristics peaks are given in table 3.7 and 3.8.

3.1.13 Thermal analytical profile

In thermal analysis methods a property of the analyte is determined as a function of an externally applied temperature¹². The temperature of the sample is increased in a linear fashion while the property in question is evaluated on a continuous basis. Thermal methods can be extremely important in preformulation studies, since the carefully planned studies are used to indicate the existence of possible drug excipients interactions in a prototype formulation¹³.

Differential Scanning Calorimetry

Differential scanning calorimetry measures the heat loss or gain resulting from physical or chemical changes within a sample as a function of temperature. Examples of endothermic (heat absorbing) process are fusion, boiling, sublimation, vaporization, desolvation, solid-solid transitions and chemical degradation. Crystallization and degradation are usually exothermic

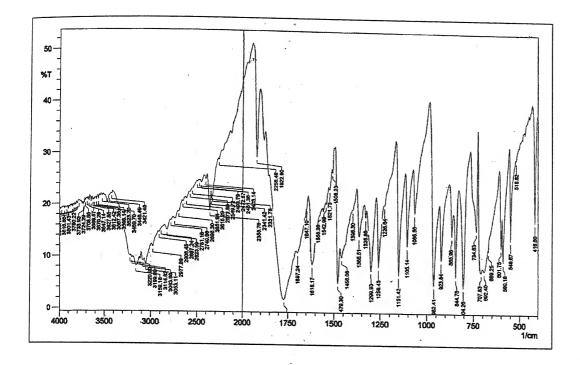
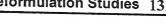


Fig. 3.7: FTIR spectrum of chlorzoxazone

Table 3.7: Principle peaks of FTIR spectrum and data analysis of pure chlorzoxazone

S.No.	Wave No. (Peak) in cm ⁻¹	Interpretation	
1.	3470	N-H stretch	
2.	3155	O-H stretch in resonance structure of oxazole	
3.	3117, 3087, 3057	C-H stretch	
4.	1482-1463	C=C stretch of aromatic ring breathing	
5.	867	C-CI stretch of aromatic ring	
6.	845-805	C-H out of plane deformation of aromatic ring	



500 1/cm 1000 750

Fig. 3.8: FTIR spectrum of indomethacin

Table 3.8: Principle peaks of FTIR spectrum and data analysis of pure indomethacin

S.No.	Wave No. (Peak) in cm ⁻¹	Interpretation
1.	3400-2500	Aromatic C-H stretch carboxylic acid O-H stretch
2.	1715, 1695	C=O stretch
3.	1600	Aromatic C=C stretch
4.	1450	O-CH₃ deformation
5.	1230	(C-O) stretch plus O-H deformation
6.	925	Carboxylic O-H out of plane deformation
7.	900-600	Various C-H out of plane deformation for substituted aromatic
8.	750	C-CI

process. Quantitative measurements of these processes have many applications in preformulation studies including purity, polymorphism14, solvation¹⁵, degradation¹⁶ and excipient compatibility¹⁷⁻¹⁸.

The samples and references be kept at the same temperature and the heat flow required to maintain the equality in temperature, is measured 19. The plots from DSC obtained as the differential rate of heating (in units of watt/ second, calories/ second or joule/second) against temperature. The area under the DSC peak is directly proportional to the heat absorbed or evolved by the thermal event and integration of these peak areas yields the heat of reaction.

Differential scanning calorimetry (DSC) curves for both chlorzoxazone and indomethacin were obtained using pyres-6 DSC (Perkin Elmer, USA). Samples were prepared by placing 5 mg. of the drug substance in to an aluminum pan, which covered and crimped for analysis. Samples were desiccated over calcium chloride for 24 hours prior to assay in an effort to remove surface absorbed water. Thermograph was analyzed qualitatively by examining both the peak temperature and the endothermic transition contour. The nitrogen flow rate was 20 ml/min and the heating rate was 5°C/min over the range of 40 to 250° C. The thermographs are shown in figure 3.9 and 3.10.

3.1.14 X-ray Powder Diffraction

Random orientation of a crystal lattice in a powder sample causes the Xrays to scatter in a reproducible pattern of peak intensities of distinct angles relative to the incident beam. The pattern of diffraction is the characteristic of a specific crystalline lattice for a given compound²⁰. An amorphous form does not produce a pattern. Single crystal X-ray analysis produces a precise identification and description of a crystalline substance.

The patterns of diffraction of X-rays by the powder samples consist of a series of peaks detected at various scattering angles. These angles and their relative intensities are correlated with computed d-spacing to provide a full crystallographic characterization of the powdered samples. To measure the powder X-ray diffraction pattern, a randomly oriented powder sample is prepared so as to expose all the planes of a sample. The scattering angle is determined by slowly rotating the sample and measured the angle of diffracted X-rays with respect to the angle of incident beam.

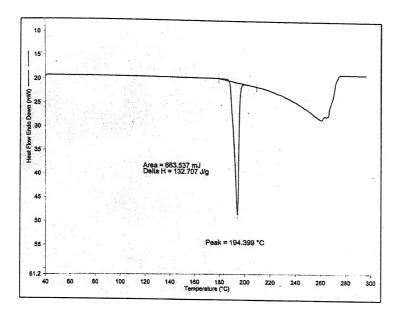


Fig. 3.9: DSC curve of chlorzoxazone

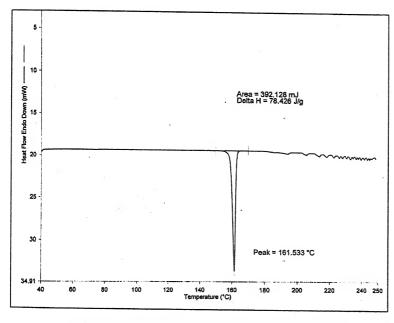


Fig. 3.10: DSC curve of indomethacin

X-ray diffraction patterns of chlorzoxazone and indomethacin were obtained at room temperature (25°C) using a D-8 Advance, Bruker-AXS Diffractometer (Germany). Samples were exposed to Cu K $\underline{\alpha}$ radiation at scanning rate 2°/min with step size 0.050°, step time 1.5 second over scanning range 3.000° to 120.000° of the diffraction angle 26; the generator was set to 40 kV and 30mA. X-ray diffraction patterns of both drugs are

shown in figure 3.11 and 3.12 and characteristic peaks are tabulated in table 3.9 and 3.10.

3.1.15 Assay

Percentage purity of indomethacin was determined by UV spectrophotometrically 21 . 10 μ g/ml solution of indomethacin in methanolic 0.1N HCl was analyzed in 1 cm cuvette using Simadzu-1701 (Japan) UV spectrophotometer at 318 nm. Percentage purity of indomethacin was calculated using extinction value $E_{1cm}^{1\%}$ as 182 at 318 nm. Percentage purity of chlorzoxazone was provided by supplier.

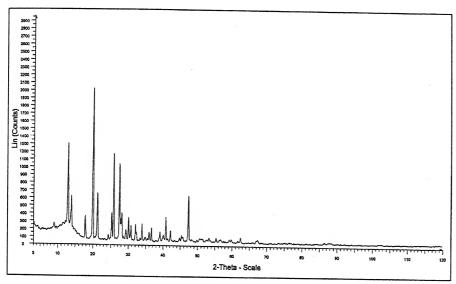


Fig. 3.11: XRPD of chlorzoxazone

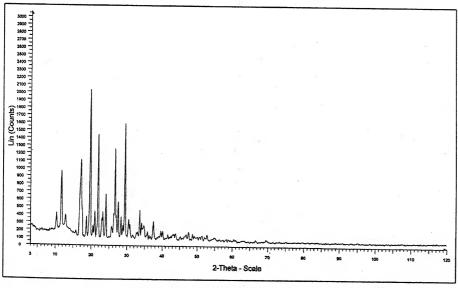


Fig. 3.12: XRPD of indomethacin

Table 3.9: X-Ray Powder Diffraction of Chlorzoxazone Cu $K\alpha$ Radiation

S.No.	2 θ (°)	d (Å)	% Intensity
1.	8.781	10.06	13.2
2.	12.746	6.94	64.3
3.	13.684	6.47	30.5
4.	17.685	5.01	17.8
5.	19.814	4.48	100.0
6.	21.107	4.21	32.7
7.	24.257	3.67	6.0
8.	25.000	3.56	11.9
9.	25.700	3.46	57.7
10.	27.461	3.25	51.3
11.	28.150	3.17	19.5
12.	29.247	3.05	9.3
13.	30.009	2.98	17.0
14.	30.650	2.91	11.9
15.	32.097	2.79	11.5
16.	33.853	2.65	13.1
17.	34.784	2.58	4.7
18.	35.900	2.50	7.9
19.	36.582	2.45	10.6
20.	38.996	2.31	8.3
21.	40.003	2.25	6.2
22.	40.770	2.21	17.5
23.	42.134	2.14	9.0
24.	45.440	1.99	5.1
25.	47.273	1.92	31.2
26.	51.260	1.78	4.4
27.	53.342	1.72	4.6
28.	55.340	1.66	4.7
29.	56.564	1.63	3.8
30.	59.177	1.56	3.9
31.	62.449	1.49	5.3
32.	67.298	1.39	3.9
33.	86.484	1.12	2.9

Table 3.10: X-Ray Powder Diffraction of Indomethacin Cu $\mbox{K}\alpha$ Radiation

S.No.	2 θ (°)	d (Å)	% Intensity
34.	10.221	8.65	20.5
35.	11.577	7.64	47.3
36.	12.749	6.94	19.1
37.	16.969	5.22	52.9
38.	18.550	4.78	18.1
39.	19.550	4.54	100.0
40.	20.774	4.27	19.5
41.	21.806	4.07	70.9
42.	23.029	3.86	19.0
43.	24.027	3.70	32.5
44.	26.581	3.35	61.8
45.	27.450	3.25	27.4
4 6.	28.351	3.15	18.1
47.	29.336	3.04	78.2
48.	30.553	2.92	14.6
49.	32.750	2.73	8.0
50.	33.594	2.67	22.5
51.	34.469	2.60	11.1
52.	37.510	2.40	15.4
53.	40.003	2.25	9.4
54.	43.708	2.07	7.8
55.	45.147	2.01	6.0
56.	46.650	1.95	6.7
57.	47.471	1.91	8.8
58.	48.836	1.86	6.3
59.	52.806	1.73	7.1
60.	54.957	1.67	5.2
61.	60.611	1.53	4.0
62.	63.270	1.47	3.8
63.	66.651	1.40	4.1
64.	69.698	1.35	4.0

3.2 METHOD OF ANALYSIS

The search for an appropriate reproducible and simple analytical method is a major test for estimation of drug in various biological and non-biological fluids selection of a particular analytical method is usually based on considerations such as feasibility, accuracy, reproducibility, convenience and suitability for the purpose.

3.2.1 Spectrophotometric Estimation of Chlorzoxazone

A spectrophotometric method as reported by Kirschner²² was used for the estimation of chlorzoxazone. The chlorzoxazone was dried at 105°C to a constant weight and stored in desiccator, protected from moisture.

3.2.1.1 Preparation of stock solution

Chlorzoxazone (50 mg) was accurately weighed and dissolved in methanol in 10 ml volumetric flask and volume was made upto 10 ml with methanol. A working standard solution (100 μ g/ml) was obtained by diluting 1 ml of this solution to 50 ml by distilled water and/or methanol (q.s. to prevent ppt) in a volumetric flask.

3.2.1.2 λ_{max} Determination

For determination of λ_{max} 1.0 ml of stock solution was diluted to 10 ml and then scanned from 200-400mm using Shimadzu 1701 spectrophotometer. The scan is shown in figure 3.5.

3.2.1.3 Construction of calibration curve

The aliquots of 0.25, 0.50, 0.75, 1.00, 1.25, 1.50. 1.75, 2.00, 2.25 and 2.50 ml of stock solution (100 μ g/ml) were transferred quantitatively in to a series of 10 ml volumetric flasks and volume was made up to 10 ml with distilled water to produce solution of concentration ranging 2.5 to 25 μ g/ml. The absorbance of these solution were determination at λ_{max} (280.0 nm) against distilled water as blank observations were recorded in table 3.11. The data were linearly regressed and various statistical parameter were calculated. The linearly regressed curve is shown in figure 3.13.

Table 3.11: Calibration curve data of chlorzoxazone in distilled water at λ_{max} 280.0 nm

S.No.	Concentration (μg/ml)	Observed Absorbance	Regressed Absorbance
1.	2.5	0.081	0.077
2.	5	0.159	0.158
3.	7.5	0.243	0.239
4.	10	0.316	0.320
5.	12.5	0.4	0.402
6.	15	0.472	0.483
7.	17.5	0.567	0.564
8.	20	0.642	0.645
9.	22.5	0.731	0.727
10.	25	0.813	0.808

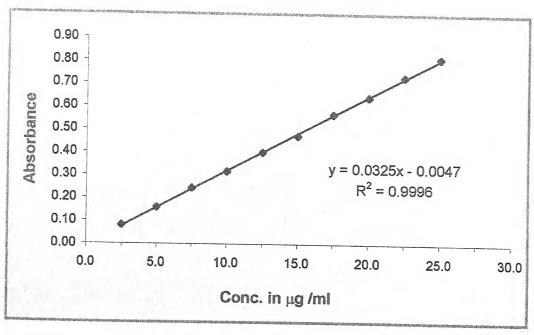


Fig. 3.13: Linearly regressed calibration curve of chlorzoxazone in distilled water at λ_{max} 280.0 nm

3.2.2 Spectrophotometric Estimation of Indomethacin

A spectrophotometric method²³ was used for the estimation of indomethacin. The indomethacin was dried at 105°C to a constant weight and stored in desiccator, protected from moisture.

3.2.2.1 Preparation of stock solution

Indomethacin (50 mg) was accurately weight and dissolved in methanol in 10 ml volumetric flask and volume was made upto 10 ml with methanol. A working standard solution (100 μ g/ml) was obtained by diluting 1 ml of this solution to 50 ml by distilled water and/or methanol (q.s. to prevent ppt) in a volumetric flask.

3.2.2.2 λ_{max} Determination

For determination of λ_{max} 1.0 ml of stock solution was diluted to 10 ml and then scanned from 200-400mm using Simadzu 1701 spectrophotometer. The scan is shown in figure 3.6.

3.2.2.3 Construction of calibration curve

The aliquots of 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6, and 4.0 ml of stock solution (100 μ g/ml) were transferred quantitatively in to a series of 10 ml volumetric flasks and volume was made up to 10 ml with distilled water to produce solution of concentration ranging 4 to 40 mg/ml. The absorbance of these solution were determination at λ_{max} (319.5 nm) against distilled water as blank observations were recorded in table 3.12. The data were linearly regressed and various statistical parameters were calculated. The linearly regressed curve is shown in figure 3.14.

Table 3.12: Calibration curve data of indomethacin in distilled water at λ_{max} 319.5 nm

S.No.	Concentration (μg/ml)	Observed Absorbance	Regressed Absorbance
1.	4	0.083	0.082
2.	8	0.171	0.163
3.	12	0.242	0.245
4.	16	0.327	0.326
5.	20	0.398	0.407
6.	24	0.491	0.489
7.	28	0.569	0.570
8.	32	0.643	0.652
9.	36	0.729	0.733
10.	40	0.828	0.815

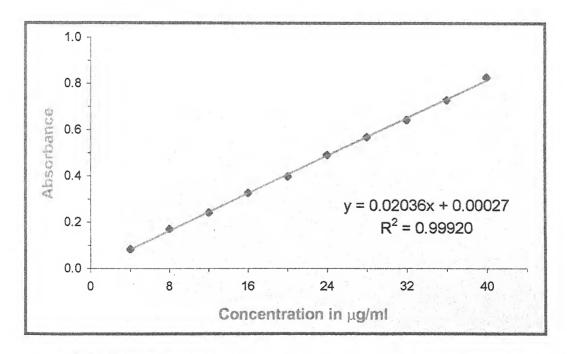


Fig. 3.14: Linearly regressed calibration curve of indomethacin in distilled water at λ_{max} 319.5 nm

Table 3.13: Optical Characteristics of Chlorzoxazone

λ_{max} (nm)	280.0
Beer's law limit (μg/ml)	2.5-25
Molar absorptivity	5.507x10 ³
Regression equation	Y = 0.0325x - 0.0047
Slope	0.0325
Intercept	- 0.0047
Correlation coefficient (r ²)	0.9996

Table 3.14: Optical Characteristics of Indomethacin

λ_{max} (nm)	319.5
Beer's law limit (μg/ml)	4-40
Molar absorptivity	7.285 x10 ³
Regression equation	Y = 0.02036x + 0.00027
Slope	0.02036
Intercept	0.00027
Correlation coefficient (r ²)	0.99920

3.3 RESULTS AND DISCUSSION

The gift sample of chlorzoxazone and indomethacin was obtained from M/s Signa Pharma Pvt. Ltd., Kanpur (U.P.), India. Both the drugs were identified and characterized as shown in the preformulation worksheets 1 and 2.

Physical appearance and meting point of the drug sample under investigation were found to be concordant with the reported values. Particle size of both drugs was determined microscopically using calibrated ocular micrometer and found to be $83.48\pm9.36~\mu m$ and $68.23\pm11.56~\mu m$ for chlorzoxazone and indomethacin respectively. Hygroscopicity of the drugs was determined at various humidity conditions in sealed desiccators of well-defined humidity conditions (Table 3.1). Both the drugs were classified as non-hygroscopic⁸, because of negligible amount of moisture was gained by drugs when kept at RH below 90% and total moisture gained was less than 20% when stored at RH 90% or above 90% for one week.

Lipophilicity of chlorzoxazone and indomethacin were determined as log P value (octanol/water partition coefficient) and found to be 1.938±0.296 and 3.172±0.258 respectively. Preliminary solubility study was conducted for both the in various pure solvents. The solubility study indicated that both the drug chlorzoxazone and indomethacin were practically insoluble in water. Chlorzoxazone was sparingly soluble in methanol and phosphate buffer (pH 7.0), while soluble in chloroform, DMSO, DMF and freely soluble in sodium hydroxide solution and ammonia solution. Indomethacin was found soluble in methanol, ethanol, butanol, DMSO, DMF and freely soluble in sodium hydroxide solution.

To find out the influence of the pH on the solubility of chlorzoxazone and indomethacin, the pH dependent solubility studies were carried out at

different pH ranging from 2.5-12.0 at 25±2°C and 35±2°C. The solubility of both the drugs were found to increase on increasing the pH of the solution (Fig. 3.1 and 3.2). The solubility of chlorzoxazone was increase to 39.722±1.5 mg/ml at pH 12.08 i.e. 124.787 fold that of aqueous solubility at 25°C. The equilibrium solubility of indomethacin was found to 3.6399±0.3085 mg/ml at pH 7.96 i.e. 145.76 times of its aqueous solubility at 25°C. The pH of saturated solution of indomethacin was not increased above 7.96 because of buffering effect of indomethacin as it is a weak acid.

Hydrolysis profiles of both the drugs were obtained at pH 3.0, 7.4 and 10.0. Semilogrithmic plots of concentration remaining versus time were linear indicating that the reaction was first order with respect to the drugs. The apparent first order hydrolysis rate constants determined from the slope of such plots are shown in table 3.6. Both the drugs were stable at lower to neutral pH, while degradation rate constant were found greater at pH 10.0 for chlorzoxazone and indomethacin.

The UV spectrum of chlorzoxazone and indomethacin was obtained by scanning 10 μ g/ml solution in distilled water of respective drug between 200-400nm using Shimadzu-1701 (Japan) UV spectrophotometer. The λ_{max} was found at 280 nm for chlorzoxazone and 265 nm and 319.5 nm for indomethacin. FTIR spectrum of both the drugs were obtained by potassium bromide disc method using FTIR-8400s Shimadzu (Japan). The principle peaks of both the drugs were identified and matched with the standard FTIR of the respective drugs, confirming identity and purity of the drug. Respective spectrum of chlorzoxazone and indomethacin are shown in figure 3.7 and 3.8 and characteristic peaks are given in table 3.7 and 3.8.

Thermal behaviour of both drugs were determined by differential scanning calorimetry (DSC) curves. The thermographs were shown in

figures 3.9 and 3.10 for chlorzoxazone and indomethacin respectively. The endothermic peak was obtained at 194.399°C and energy for endothermic transition (ΔH) was 132.707 J/g for chlorzoxazone which was attributed to its melting. The endothermic peak for indomethacin was obtained at 161.533°C attributed to its melting point and energy for its endothermic transition was 78.426 J/g.

Crystal properties of both drugs were determined by performing X-ray powder diffraction (XRPD). Chlorzoxazone showed characteristic diffraction peaks at 8.781, 12.746, 19.814, 24.257, 25.700 and 27.461° at 20 scale (Fig. 3.11), confirming that the drug was a high quality crystalline solid²⁴. Indomethacin showed characteristic diffraction peaks at 11.577, 16.969, 19.55, 21.806, 26.581 and 29.336°+ at 20 scale (Fig. 3.12), attributed to γ form of indomethacin²⁵⁻²⁶, indicating that the bulk powder was a high quality crystalline solid.

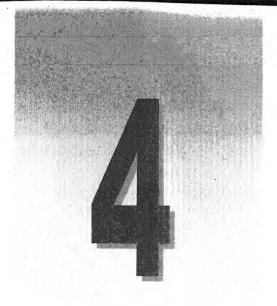
Percentage purity of both drugs was determined by performing assay by reported procedure and found to be 99.68% and 99.47% for chlorzoxazone and indomethacin respectively.

Standard calibration curves of chlorzoxazone and indomethacin are prepared in distilled water at their respective λ_{max} i.e. 280.0 and 319.5nm respectively. The optical characteristics of the curves are shown in table 3.13 and 3.14.

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Solubility Studies

- 4.1 Hydrotropic Solubilization
- 4.2 Complexation Solubilization
- 4.3 Cosolvent Solubilization
- 4.4 Surfactant Solubilization

Recent development in the field of Drug delivery technology has revolutionized the therapies of the several clinical disorders. Though Novel Drug design and Drug delivery system has become a fashionable catch word today, but it should not be forgotten that, this is only beginning and there are greater challenges still to met. At the same time, traditional methods after good promise of making convenient dosage forms with greater bioavailability. One of the major concerns is increasing the water solubility of insoluble and poorly soluble drugs.

Solubility of organic compounds is in general of Pharmaceutical interest because it has been recognized as a key factors in pharmacological profile of a drug its chemical stability and ultimately its formulation.

The Objective of this study is to enhance the solubility for injectable delivery of two water insoluble drugs chlorzoxazone and indomethacin that minimize risk and allow the formulation of a stable, sterilized product.

Attempt has been documented in the literature for improving the physicochemical properties of a wide variety of compounds, in hope of improving their aqueous solubility for parenteral formulations through hydrotropic and cosolvent solubilization approaches. The present work explores the utility of

- Hydrotropic solubilization
- 2. Complexation solubilization
- 3. Co-solvent solubilization
- 4. Surfactant solubilization

4.1 HYDROTROPIC SOLUBILIZATION

Increase the water solubility of insoluble and slightly soluble drugs is of major concern. Addition of hydrotropes or hydrotropic agents is one aqueous solubilization technique and the term hydrotropic agent was first introduced by Neuberg¹ to designate anionic organic salts, which at high concentrations, considerably increase the aqueous solubility of poorly soluble solutes. This is in contrast to "normal" solution behaviour since addition of a second compound, especially at high concentrations, generally causes precipitation of the less-soluble solute. Saleh El-Khordagui² extended the definition of the

term hydrotropic agent to include cationic and non-ionic organic compounds bearing the essential structural features of Neuberg's hydrotropes.

Hydrotropic Solubilization is a method suitable for parenteral formulations. Addition of these solubility modifiers should be in minimum concentration so that minimum tissue toxicity and low hemolytic effect would observe. Hydrotropic solubilization is advantageous in comparison to co solvent solubilization in respect of minimizing risk of precipitation on dilution with body fluid or I.V. fluids. However, hemolytic behaviors up to some extent can be adjusted by tonicity modifier. Although hydrotropic solubilization is suitable to parenteral formulation but care must be taken that these hydrotropes must be non toxic and do not accumulate in the body. The concentration of these hydrotrope must be minimum.

4.1.1 Selection of Hydrotrope

On the basis of previous report and qualitative study five hydrotropes were selected for the present study namely nicotinamide, sodium benzoate, sodium p-hydroxy benzoate, resorcinol and urea. Nicotinamide, vitamin B₃, is well-known as a hydrotropic agent and has demonstrated the ability to solubilize a wide variety of therapeutic entities³⁻¹¹. Urea, widely known as a protein denaturant, is also known to have some hydrotropic properties. Although its hydrotropic ability has not been studied as thoroughly as that of nicotinamide, it has demonstrated an ability to solubilize a wide array of compounds including aminophenazone, thiazide diuretics, acetazolamide and the structural isomers of hydroxybenzoic acid^{3, 12, 13}.

4.1.2 Identification and Characterization of Hydrotropes

4.1.2.1 FTIR spectral studies

The FTIR spectrum of each hydrotrope i.e. nicotinamide, sodium benzoate, sodium p-hydroxy benzoate, resorcinol and urea were obtained by means of FTIR spectrophotometer (FTIR-8400s) using potassium bromide disc method and matched with the respective reference FTIR spectrum. These are presented in figure 4.1.1 to 4.1.5.

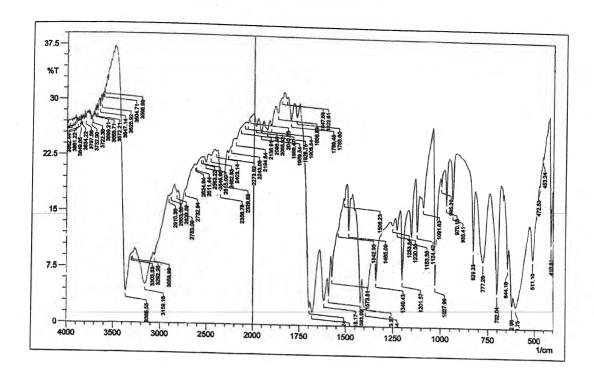


Fig. 4.1.1: FTIR spectrum of nicotinamide

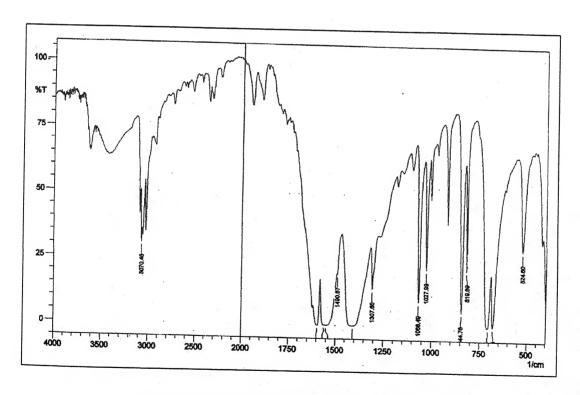


Fig. 4.1.2: FTIR spectrum of sodium benzoate

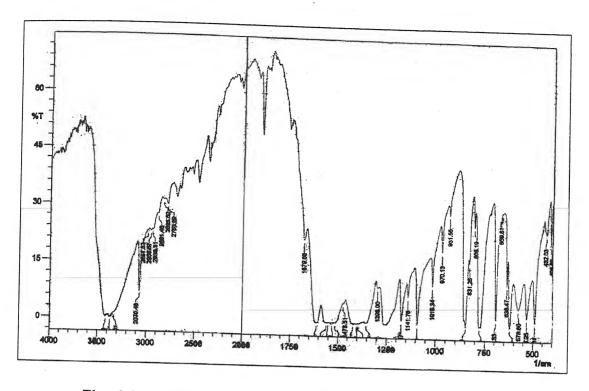


Fig. 4.1.3: FTIR spectrum of sodium p-hydroxy benzoate

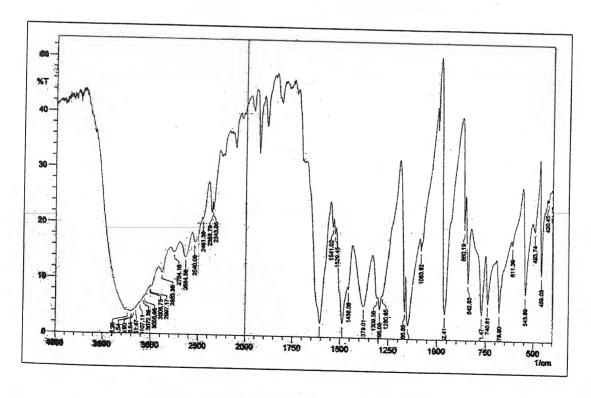


Fig. 4.1.4: FTIR spectrum of resorcinol

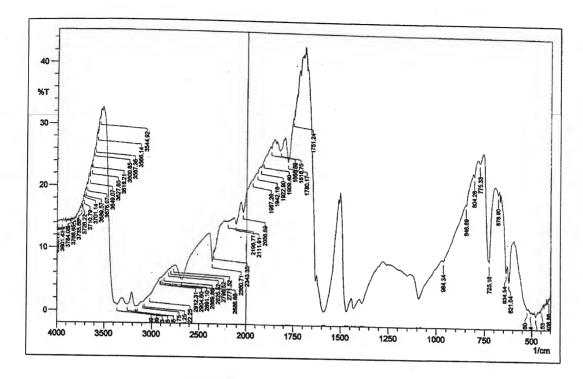


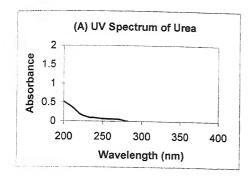
Fig. 4.1.5: FTIR spectrum of urea

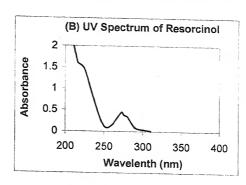
4.1.2.2 UV spectral studies

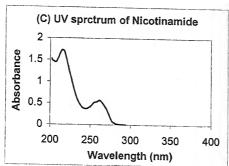
UV spectral studies of the hydrotrope were conducted in order to study interference, if any in the absorbance of drugs due to the presence of hydrotrope.

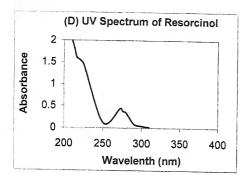
Determination of λ_{max} of hydrotrope

Stock solutions of 10 mg/ml of each hydrotrope i.e. nicotinamide, sodium benzoate, sodium p-hydroxy benzoate, resorcinol and urea were prepared by dissolving accurately weighed quantity of the respective hydrotrope. These solutions were scanned between 200-400 nm by UV spectrophotometer. The values of λ_{max} of these hydrotropes are shown in table 4.1.1 and spectra are presented in figure 4.1.6 (I).









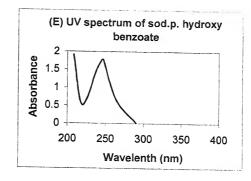


Fig. 4.1.6 (I): UV spectrum of hydrotropes

Table 4.1.1: Absorption maxima (λ_{max}) chlorzoxazone and indomethacin in hydrotropic solution

		λ_{max}			
Substance	In distilled water	With Chlorzoxazone	With Indomethacin		
Chlorzoxazone	280				
Indomethacin	265,319.5				
Urea	236.5	280.5	265,315		
Resorcinol	273.5	274.5,279.5	272.5,319.5		
Nicotinamide	215,261.5	263,269.5	261.5,319.5		
Sodium benzoate	225	235.5,279.5	223,265.5,319		
Sodium p-hydroxy benzoate	246.5	230,246.5,280	246.5,319.5		
Hp 2-Cyclodextrin	No	280	264,319.5		

4.1.3 Phase Solubility Study of Chlorzoxazone in Hydrotrope Solution

The equilibrium phase solubility experiment was performed by the method reported by Higuchi and Connors¹⁴. Hydrotrope solutions of nicotinamide, sodium benzoate, sodium p-hydroxy benzoate, resorcinol and urea of known concentration 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 M were prepared by dissolving required amount of the respective hydrotrope in water.

An excess quantity of chlorzoxazone was added to screw capped 15 ml glass culture tubes containing 10 ml of hydrotrope solution. The culture tubes were shaken vigorously for 15 minutes on touch type vortex mixer (Jyoti Scientific Industries Gwalior-474 009, India) and then the solutions were allowed to equilibrate with mechanically shaking and intermittent vortexing for 72 hrs at 25±2°C and 37±2°C in a Rotary flask shaker and shaker water bath (Jyoti scientific Industries Gwalior-474 009, India). After completion of 72 hrs, each culture tube was centrifuged for 10 min at 2000 rpm. The supernatant of each culture tube was filtered through 0.45μ membrane syringe filter (Sonar Axiva, Axiva Sichem Pvt. Ltd. Delhi, India.), filtrate diluted suitably with distilled water and analyzed spectrophotometrically at 280 nm against respective solvent system diluted accordingly as blank. The solubility of chlorzoxazone was determined in triplicate.

Solubility of chlorzoxazone in mg/ml was calculated in different hydrotropes solution of different concentration and shown in table 4.1.2-4.1.6 and graphically presented in figure 4.1.7-4.1.12.

Solubility enhancement ratio was calculated by the formula:

Solubility enhancement ratio = Solubility in hydrotrope solution

Solubility in water

Solubility enhancement ratios are reported in the same table and graphically presented in figure 4.1.13.

Table 4.1.2: Solubility data of chlorzoxazone in various concentration of urea solution

S.	Molar Concentration of -	Solubility	Solubility in mg/ml		nent Ratio
No.	Urea	At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C
1.	0.0	0.318±0.013	0.347±0.012	1.000	1.000
2.	0.2	0.355±0.028	0.386±0.036	1.115	1.112
3.	0.4	0.406±0.053	0.428±0.048	1.389	1.232
4.	0.6	0.455±0.034	0.502±0.040	1.552	1.446
5.	0.8	0.538±0.036	0.573±0.049	1.690	1.650
6.	1.0	0.594±0.055	0.639±0.044	1.866	1.840
7.	1.2	0.662±0.045	0.711±0.056	2.080	2.047

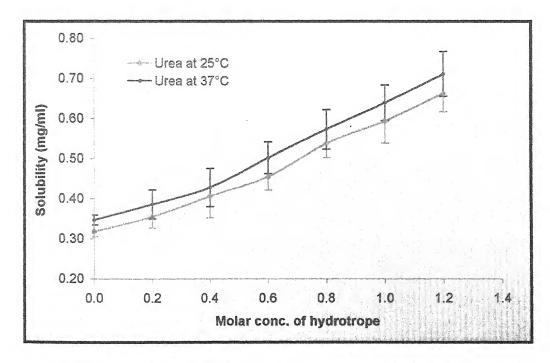


Fig. 4.1.7: Solubility plot of chlorzoxazone in various concentration of urea solution

Table 4.1.3: Solubility data of chlorzoxazone in various concentration of resorcinol solution

S.	Molar Concentration of –	in mg/ml Enhancement Ratio			
No.	Resorcinol	At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C
1.	0.0	0.318±0.013	0.347±0.012	1.000	1.000
2.	0.2	0.704±0.104	0.762±0.089	2.211	2.194
3.	0.4	1.177±0.078	1.259±0.124	3.698	3.625
4.	0.6	1.849±0.118	1.957±0.141	5.809	5.635
5.	0.8	2.580±0.110	2.616±0.103	8.105	7.533
6.	1.0	3.329±0.226	3.541±0.213	10.458	10.197
7.	1.2	4.184±0.166	4.368±0.187	13.144	12.578

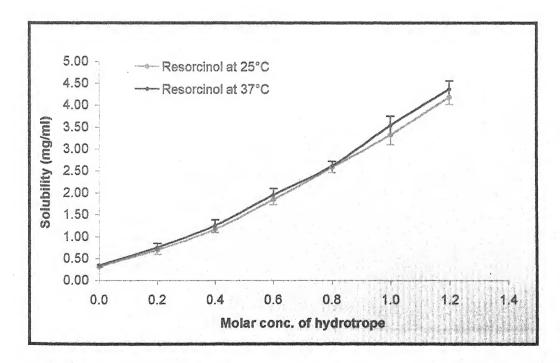


Fig. 4.1.8: Solubility plot of chlorzoxazone in various concentration of resorcinol solution

Table 4.1.4: Solubility data of chlorzoxazone in various concentration of nicotinamide solution

S.	Molar Concentration of – Nicotinamide	Solubility	in mg/ml	Enhancement Ratio	
No.		At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C
1.	0.0	0.318±0.013	0.347±0.012	1.000	1.000
2.	0.2	2.174±0.168	2.296±0.176	6.829	6.612
3.	0.4	3.873±0.276	4.210±0.291	12.167	12.123
4.	0.6	5.771±0.253	6.378±0.360	18.130	18.366
5.	0.8	7.839±0.337	8.292±0.406	24.626	23.878
6.	1.0	10.043±0.460	10.652±0.491	31.550	30.674
7.	1.2	12.225±0.529	13.112±0.775	38.405	37.757

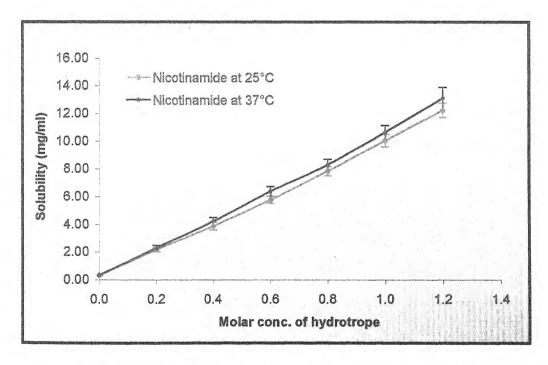


Fig. 4.1.9: Solubility plot of chlorzoxazone in various concentration of nicotinamide solution

Table 4.1.5: Solubility data of chlorzoxazone in various concentration of sodium benzoate solution

S. No.	Molar Concentration of - Sodium benzoate	Solubility in mg/ml		Enhancement Ratio	
		At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C
1.	0.0	0.318±0.013	0.347±0.012	1.000	1.000
2.	0.2	2.338±0.154	2.452±0.122	7.345	7.061
3.	0.4	4.563±0.243	4.864±0.184	14.335	14.006
4.	0.6	7.345±0.326	7.916±0.364	23.074	22.795
5.	0.8	9.655±0.386	10.125±0.410	30.331	29.156
6.	1.0	13.334±0.527	14.225±0.483	41.889	40.962
7.	1.2	17.215±0.812	18.564±0.745	54.081	53.457

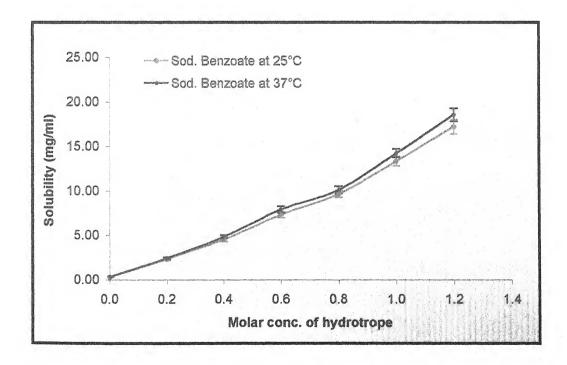


Fig. 4.1.10: Solubility plot of chlorzoxazone in various concentration of sodium benzoate solution

Table 4.1.6: Solubility data of chlorzoxazone in various concentration of sodium p-hydroxy benzoate solution

S. No.	Molar Concentration of Sodium p- hydroxy benzoate	Solubility in mg/ml		Enhancement Ratio	
		At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C
1.	0.0	0.318±0.013	0.347±0.012	1.000	1.000
2.	0.2	2.412±0.089	2.641±0.110	7.577	7.605
3.	0.4	4.768±0.148	4.992±0.099	14.979	14.375
4.	0.6	7.684±0.267	8.458±0.251	24.139	24.356
5.	8.0	10.115±0.316	10.534±0.453	31.776	30.334
6.	1.0	14.234±0.527	14.863±0.286	44.716	42.800
7.	1.2	18.355±0.618	19.485±0.542	57.662	56.109

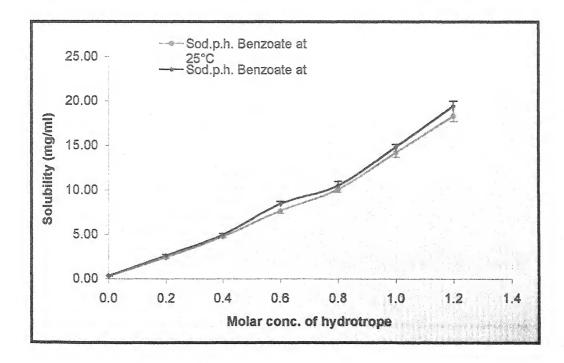


Fig. 4.1.11: Solubility plot of chlorzoxazone in various concentration of sodium p-hydroxy benzoate solution

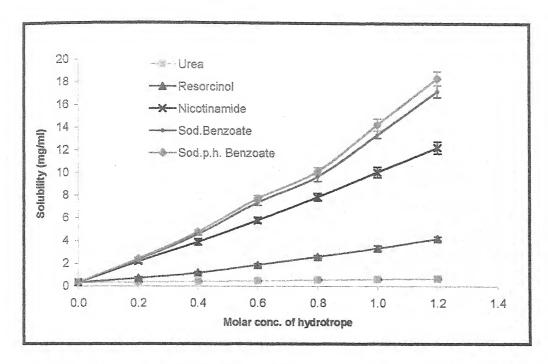


Fig. 4.1.12: Comparative solubility plot of chlorzoxazone in various concentration of hydrotropes at 25°C

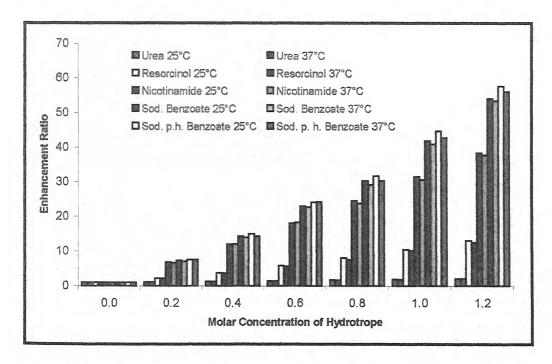


Fig. 4.1.13: Solubility enhancement plot of chlorzoxazone in various concentration of hydrotropes

4.1.4 Phase Solubility Study of Indomethacin in Hydrotrope Solution

The equilibrium phase solubility experiment of indomethacin was performed by the same method as used for chlorzoxazone. Hydrotrope solutions were prepared as above. An excess quantity of indomethacin was equilibrated with the hydrotrope solutions for 72 hrs at 25±2°C and 37±2°C then centrifuged and filtered through 0.45 μ membrane syringe filter, The filtrate diluted suitably with distilled water and analyzed spectrophotometrically at 319.5 nm against respective solvent system diluted accordingly as blank. The solubility of Indomethacin was determined in triplicate.

Solubility of indomethacin in mg/ml was calculated in different hydrotropes solution of different concentration and shown in table 4.1.7-4.1.11 and graphically presented in figure 4.1.14-4.1.19.

Solubility enhancement ratio was also calculated and reported in the same tables and graphically presented in figure 4.1.20.

4.1.5 Mechanistic Studies

In order to understand and interpret probable mechanism of hydrotropic solubilization various properties of hydrotropic solution, UV, spectral studies, FTIR studies, thermal analysis and XRPD were performed.

4.1.5.1 Determination of Solution Properties of Different Hydrotrope Solution

Various solution properties such as pH, viscosity, specific gravity, surface tension, refractive index and conductance of different hydrotrope solutions were studies at 25±2°C. These properties were studied in order to deduce the mechanism of solubilization.

pН

The pH of different hydrotrope solution at concentrations 0.2 to 2.0 M was measured at 25±2°C using pH meter. The results are reported in table 4.1.12.

Specific Gravity

The specific gravity of different hydrotrope solutions 0.2 to 2.0 M determined at $25\pm2^{\circ}$ C using water as reference using pycnometer, using following formula¹⁵.

Table 4.1.7: Solubility data of indomethacin in various concentration of urea solution

S. No.	Molar Concentration - of Urea	Solubility in mg/ml		Enhancement Ratio	
		At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C
1.	0.0	0.025 ± 0.005	0.028 ± 0.007	1.000	1.000
2.	0.2	0.033 ± 0.013	0.037 ± 0.011	1.321	1.303
3.	0.4	0.043 ± 0.031	0.046 ± 0.022	1.722	1.620
4.	0.6	0.058 ± 0.011	0.061 ± 0.014	2.323	2.148
5.	0.8	0.079 ± 0.013	0.083 ± 0.017	3.164	2.923
6.	1.0	0.099 ± 0.012	0.104 ± 0.015	3.964	3.663
7.	1.2	0.117 ± 0.013	0.126 ± 0.029	4.685	4.438
8.	2.0	0.232 ± 0.027	0.235 ± 0.072	9.291	8.275

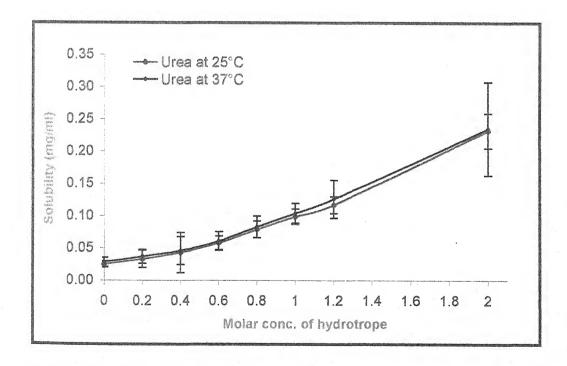


Fig. 4.1.14: Solubility plot of indomethacin in various concentration of urea solution

Table 4.1.8: Solubility data of indomethacin in various concentration of resorcinol solution

S.	Molar	Solubility	in mg/ml	Enhancen	Enhancement Ratio	
No.	Concentration of Urea	At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C	
1.	0.0	0.025 ± 0.005	0.028 ± 0.007	1.000	1.000	
2.	0.2	0.038 ± 0.011	0.041 ± 0.012	1.522	1.451	
3.	0.4	0.062 ± 0.027	0.069 ± 0.019	2.463	2.427	
4.	0.6	0.096 ± 0.028	0.106 ± 0.031	3.860	3.723	
5.	0.8	0.149 ± 0.044	0.166 ± 0.044	5.951	5.840	
6.	1.0	0.222 ± 0.033	0.244 ± 0.041	8.874	8.576	
7.	1.2	0.327 ± 0.033	0.362 ± 0.058	13.095	12.764	
8.	2.0	0.750 ± 0.103	0.818 ± 0.100	30.043	28.816	

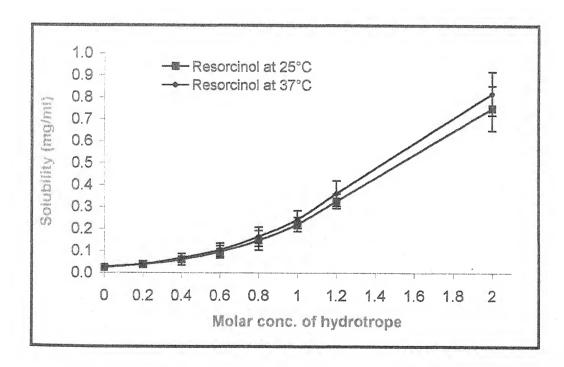


Fig. 4.1.15: Solubility plot of indomethacin in various concentration of resorcinol solution

Table 4.1.9: Solubility data of indomethacin in various concentration of nicotinamide solution

NIA	Molar	Solubility	in mg/ml	Enhancement Ratio	
	Concentration of Urea	At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C
1.	0.0	0.025 ± 0.005	0.028 ± 0.007	1.000	1.000
2.	0.2	0.073 ± 0.023	0.079 ± 0.038	2.939	2.782
3.	0.4	0.134 ± 0.024	0.148 ± 0.027	5.366	5.213
4.	0.6	0.229 ± 0.042	0.257 ± 0.026	9.170	9.052
5.	0.8	0.376 ± 0.078	0.412 ± 0.140	15.057	14.511
6.	1.0	0.582 ± 0.117	0.637 ± 0.306	23.306	22.435
7.	1.2	0.775 ± 0.343	0.827 ± 0.226	31.035	29.127
8.	2.0	1.725 ± 0.171	1.882 ± 0.210	69.065	66.278

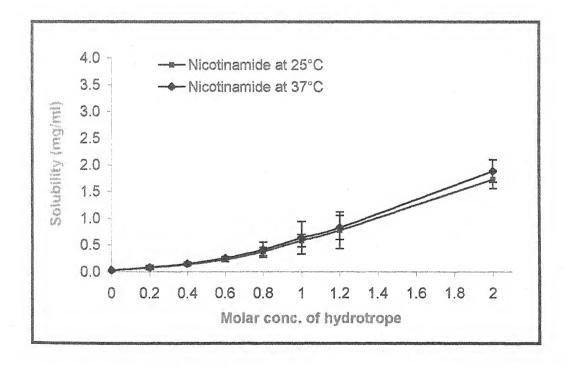


Fig. 4.1.16: Solubility plot of indomethacin in various concentration of nicotinamide solution

Table 4.1.10: Solubility data of indomethacin in various concentration of sodium benzoate solution

No Cond	Molar Concentration	Solubility	in mg/ml	Enhancement Ratio	
	of Urea	At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C
1.	0.0	0.025 ± 0.005	0.028 ± 0.007	1.000	1.000
2.	0.2	0.084 ± 0.053	0.091 ± 0.054	3.364	3.205
3.	0.4	0.154 ± 0.145	0.172 ± 0.134	6.167	6.058
4.	0.6	0.261 ± 0.186	0.286 ± 0.248	10.452	10.073
5.	0.8	0.417 ± 0.302	0.451 ± 0.186	16.699	15.884
6.	1.0	0.657 ± 0.276	0.702 ± 0.191	26.309	24.725
7.	1.2	0.963 ± 0.232	0.991 ± 0.281	38.563	34.903
8.	2.0	2.000 ± 0.227	2.142 ± 0.223	80.076	75.442

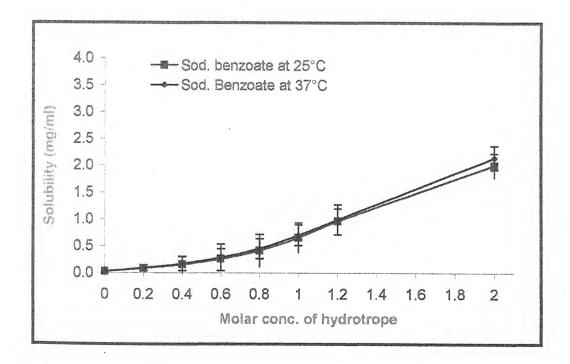


Fig. 4.1.17: Solubility plot of indomethacin in various concentration of sodium benzoate solution

Table 4.1.11: Solubility data of indomethacin in various concentration of sodium p-hydroxy benzoate solution

S.	Molar Concentration	Solubility	in mg/ml	Enhancen	Enhancement Ratio	
No.	of Urea	At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C	
1.	0.0	0.025 ± 0.005	0.028 ± 0.007	1.000	1.000	
2.	0.2	0.092 ± 0.043	0.099 ± 0.027	3.700	3.483	
3.	0.4	0.178 ± 0.062	0.189 ± 0.043	7.128	6.657	
4.	0.6	0.316 ± 0.085	0.338 ± 0.023	12.638	11.919	
5.	0.8	0.528 ± 0.068	0.553 ± 0.126	21.144	19.480	
6.	1.0	0.847 ± 0.105	0.887 ± 0.106	33.906	31.230	
7.	1.2	1.246 ± 0.221	1.315 ± 0.337	49.895	46.329	
8.	2.0	2.934 ± 0.435	3.115 ± 0.310	117.499	109.711	

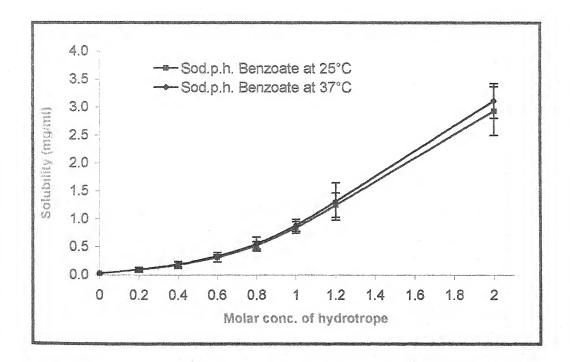


Fig. 4.1.18: Solubility plot of indomethacin in various concentration of sodium p-hydroxy benzoate solution

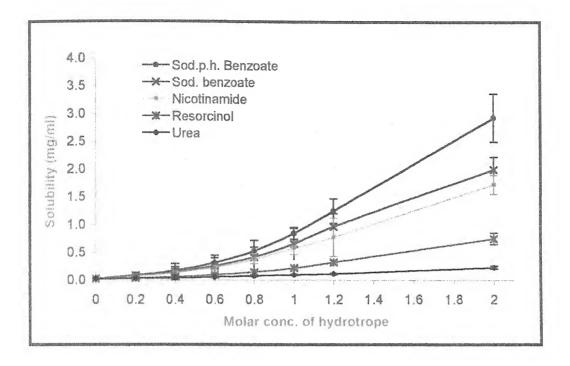


Fig. 4.1.19: Comparative solubility plot of indomethacin in various concentration of hydrotropes

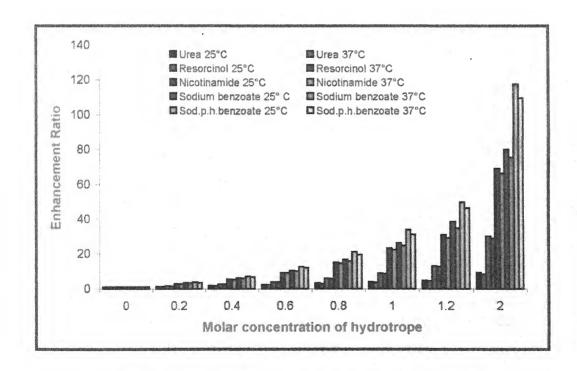


Fig. 4.1.20: Solubility enhancement plot of indomethacin in various concentration of hydrotropes

Table 4.1.12: pH of hydrotrope solution alone and saturated with drug

	Molar	рН					
S.No.	Conc. of hydrotrope	Urea	Nicotinmide	Sodium benzoate	Sodium p-hydroxy benzoate	Resorcinol	
			Witho	out Drug			
1.	0.2	7.08	6.70	6.81	5.20	4.67	
2.	0.4	7.29	6.56	6.93	5.38	4.32	
3.	0.6	7.45	6.51	7.16	5.69	4.03	
4.	8.0	7.76	6.42	7.34	5.73	3.77	
5.	1.0	7.90	6.15	7.57	5.84	3.51	
6.	1.2	8.03	5.94	7.83	5.98	3.28	
7.	2.0	8.67	5.48	8.02	6.12	2.86	
		,	Saturated with	Chlorzoxa	zone		
8.	0.2	6.89	6.73	6.74	6.01	5.38	
9.	0.4	6.87	6.68	6.78	6.13	5.26	
10.	0.6	6.92	6.64	6.81	6.27	5.12	
11.	0.8	7.01	6.54	6.88	6.33	4.89	
12.	1.0	7.05	6.45	7.12	6.42	4.67	
13.	1.2	7.09	6.34	7.21	6.48	4.51	
14.	2.0	7.16	6.12	7.34	6.67	4.22	
		Sat	turated with In	domethacir	1		
15.	0.2	6.82	6.34	5.68	5.11	5.81	
16.	0.4	6.53	5.89	5.86	5.27	5.73	
17.	0.6	6.49	5.72	5.95	5.34	5.36	
18.	0.8	6.51	5.49	6.15	5.4	5.05	
19.	1.0	6.57	5.46	6.57	5.53	4.96	
20.	1.2	6.59	5.41	6.84	5.75	4.84	
21.	2.0	6.72	5.13	7.01	6.1	3.97	

Specific Gravity =
$$\frac{W_3 - W_1}{W_2 - W_1}$$

Where W_1 = Weight of empty pycnometer; W_2 = Weight of pycnometer + water; W_3 = Weight of pycnometer + hydrotrope solution. The observation are recorded in table 4.1.13 and shown graphically in figure 4.1.21.

Viscosity

The viscosity of different hydrotrope solutions (0.2 to 2.0 M) was determined using water as reference at 25±2°C by Ostwald Viscometer. The viscosity was calculated using following equation¹⁵.

$$\eta_1 = \frac{\rho_1 t_1}{\rho_2 t_2} \times \eta_2$$

Where, η_1 and η_2 are viscosities, ρ_1 and ρ_2 are densities and t_1 and t_2 are times required for the flow of unknown hydrotrope solution and reference liquid, respectively. The values are recorded in table 4.1.14 and presented graphically in figure 4.1.22.

Surface Tension

The surface tension of different hydrotrope solutions (0.2 to 2.0 M) was determined using water as references at 25±2°C using Stalagmometer. The surface tension was calculated using following equation¹⁵.

$$\gamma_1 = \frac{\rho_1 n_1}{\rho_2 n_2} \times \gamma_2$$

Where, γ_1 and γ_2 are surface tension, ρ_1 and ρ_2 are densities and n_1 and n_2 are number of drops formed of unknown hydrotrope solution and reference liquid water respectively. The values are recorded in table 4.1.15 and presented graphically in figure 4.1.23.

Refractive Index

The refractive index of different hydrotrope solutions with drug was determined at 25±2°C using Abbe refractometer. The values are reported in table 4.1.16 and presented graphically in figure 4.1.24.

Conductance

Conductivity of different hydrotrope solutions (0.2 to 2.0 M) with drug were determined using conductivity meter. The value are recorded in table 4.1.17 and presented graphically in figure 4.1.25.

Table 4.1.13: Specific gravity of hydrotrope solution in different molar concentration

Molar	Specific Gravity (gm/ml)						
Concentration of Hydrotrope	Urea	Nicotinmide	Sodium Benzoate	Sodium p-hydroxy Benzoate	Resorcinol		
0.2	1.0093	1.0032	1.0084	1.0079	1.0314		
0.4	1.0151	1.0056	1.0194	1.0134	1.0598		
0.6	1.0188	1.0083	1.0279	1.0248	1.0869		
0.8	1.0305	1.0117	1.0387	1.0311	1.1060		
1.0	1.0416	1.0142	1.0491	1.0392	1.1270		
1.2	1.0498	1.0169	1.0598	1.0431	1.1402		
2.0	1.0784	1.0228	1.0886	1.0687	1.1783		

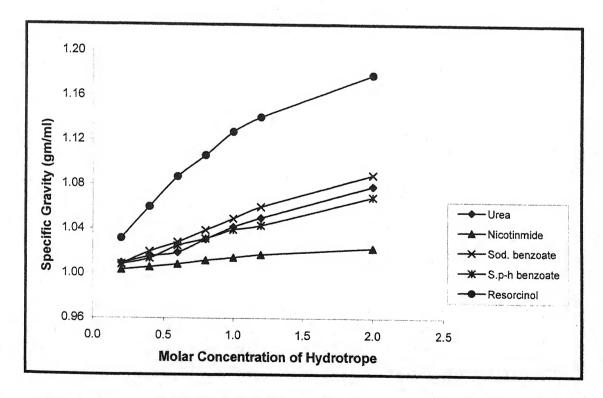


Fig. 4.1.21: Plot of Specific gravity Vs. molar hydrotrope concentration

Table 4.1.14: Viscosity of hydrotrope solution in different molar concentration

Molar		Viscosity (cps.)					
Concentration of Hydrotrope	Urea	Nicotinmide	Sodium Benzoate	Sodium p-hydroxy Benzoate	Resorcinol		
0.2	1.1781	1.1246	1.0108	0.9438	0.9965		
0.4	1.1826	1.1842	1.0654	1.0224	1.0842		
0.6	1.2092	1.2483	1.2005	1.0745	1.1237		
0.8	1.2289	1.3127	1.3086	1.1457	1.1498		
1.0	1.2533	1.4008	1.4176	1.1928	1.1675		
1.2	1.2745	1.4853	1.5124	1.2344	1.1868		
2.0	1.5098	1.8242	2.0627	1.5418	1.2946		

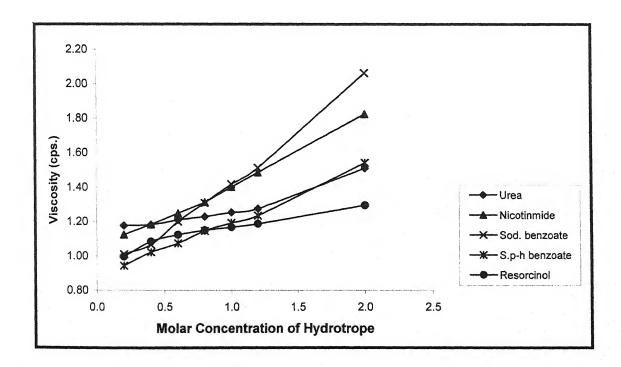


Fig. 4.1.22: Plot of viscosity Vs. molar hydrotrope concentration

Table 4.1.15: Surface tension of hydrotrope solution in different molar concentration

Molar	Surface Tension (dynes/cm)						
Concentration of Hydrotrope	Urea	Nicotinmide	Sodium Benzoate	Sodium p-hydroxy Benzoate	Resorcinol		
0.2	69.23	70.12	65.58	68.56	72.88		
0.4	67.48	68.53	58.90	64.45	66.54		
0.6	64.85	65.18	54.10	61.84	64.14		
0.8	63.42	64.66	52.64	56.30	62.38		
1.0	63.12	63.29	50.10	54.81	61.07		
1.2	64.37	61.92	50.58	54.02	62.21		
2.0	65.49	63.57	56.38	56.30	65.76		

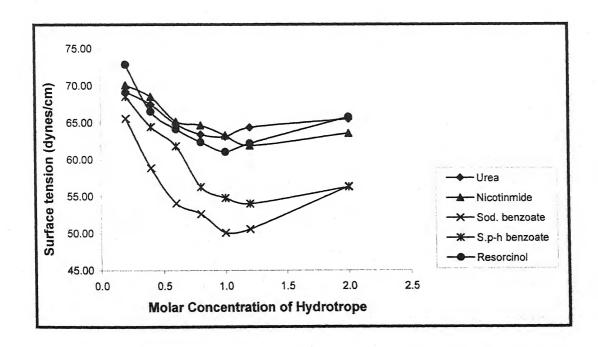


Fig. 4.1.23: Plot of surface tension Vs. molar hydrotrope concentration

Table 4.1.16: Refractive index of hydrotrope solution in different molar concentration

Molar	Refractive Index					
Concentration of Hydrotrope	Urea	Nicotinmide	Sodium Benzoate	Sodium p-hydroxy Benzoate	Resorcinol	
0.2	1.339	1.348	1.343	1.338	1.336	
0.4	1.342	1.352	1.344	1.345	1.340	
0.6	1.346	1.357	1.348	1.351	1.344	
0.8	1.349	1.361	1.352	1.362	1.349	
1.0	1.353	1.366	1.357	1.367	1.354	
1.2	1.358	1.372	1.362	1.374	1.359	
2.0	1.369	1.401	1.379	1.394	1.381	

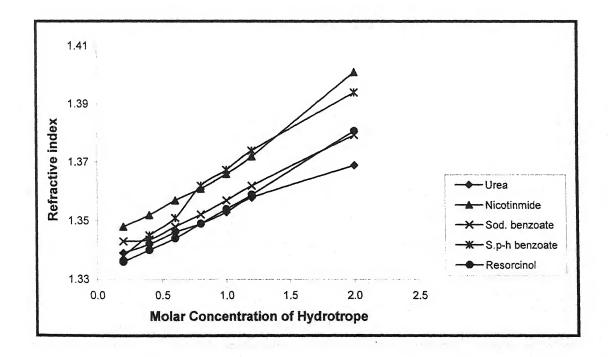


Fig. 4.1.24: Plot of refractive index Vs. molar hydrotrope concentration

Table 4.1.17: Specific conductance of hydrotrope solution in different molar concentration

Molar	Specific Conductance (10 ⁻² mho/cm)					
Concentration of Hydrotrope	Urea	Nicotinmide	Sodium Benzoate	Sodium p-hydroxy Benzoate	Resorcinol	
0.2	1.024	0.037	2.082	2.134	2.348	
0.4	1.679	0.043	2.476	2.655	2.684	
0.6	1.942	0.049	2.834	3.247	2.542	
0.8	2.315	0.051	2.983	3.686	2.567	
1.0	2.687	0.058	3.988	4.094	2.637	
1.2	3.120	0.061	4.523	4.427	2.714	
2.0	3.945	0.079	5.635	5.291	2.864	

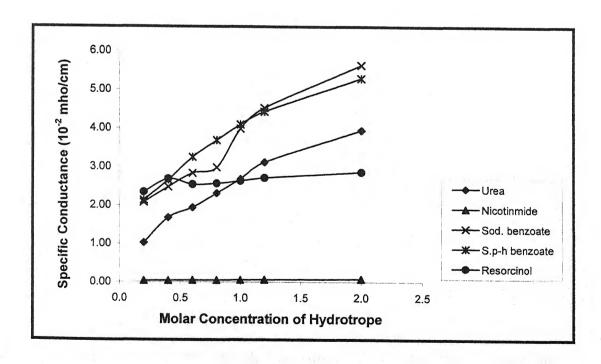


Fig. 4.1.25: Plot of specific conductance Vs. molar hydrotrope concentration

4.1.5.2 UV spectral studies

Stock solution (1 ml, 100 μ g/ml) of the drug (Section 3.2.1.1 and 3.2.2.1) was diluted to 10 ml with 10 mg/ml solution of different hydrotropes. The resultant solution was scanned in UV range from 200 to 400 nm using distilled water as blank by Simadzu-1701 (Japan) UV spectrophotometer. Any shift in λ_{max} of the drug or respective hydrotrope were noted and shown in table 4.1.1. The spectra are also recorded and presented in figure 4.1.6(II).

4.1.5.3 FTIR spectral studies

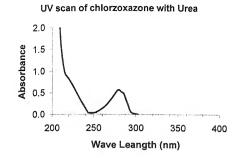
FTIR spectral studies of physical mixture of each drug with various hydrotropes and their dried solubilized product were performed using FTIR-spectrophotometer (FTIR-8400s Shimadzu, Japan). All samples were dried in vacuum for 24 hrs before FTIR studies. 5 mg of each sample was mixed with about 100 mg of potassium bromide (vacuum dried for 24 hrs) and compressed as pallet. Measurements were attempted with accumulation of 20 scans and a resolution of 4 cm⁻¹ over a range of 400 to 4000 cm⁻¹ (Fig. 4.1.26-4.1.41).

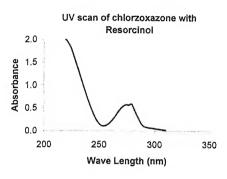
4.1.5.4 Thermal Analysis

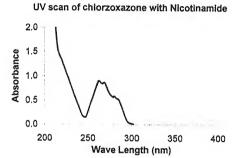
Thermal analysis of dried solubilized products and physical mixture of each drug with various hydrotropes are performed by differential scanning calorimetry, using pyres-6 DSC (Perkin Elmer, USA). Samples were prepared by placing 5 mg. of the drug substance in to an aluminum pan, which covered and crimped for analysis. Samples were desiccated over calcium chloride for 24 hours prior to assay in an effort to remove surface absorbed water. Thermograph was analyzed qualitatively by examining both the peak temperature and the endothermic transition contour. The nitrogen flow rate was 20 ml/min and the heating rate was 5°C/min over the range of 40 to 300°C/400°C. The thermographs are shown in figure 4.1.42-4.1.54.

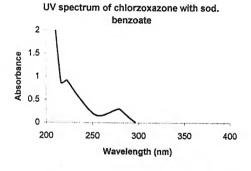
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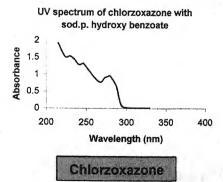


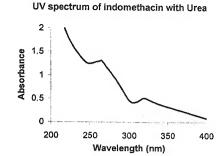


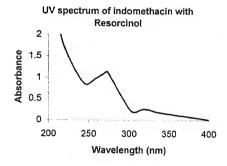


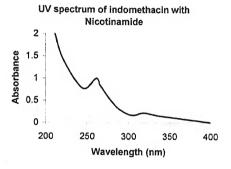


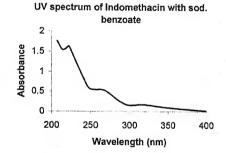












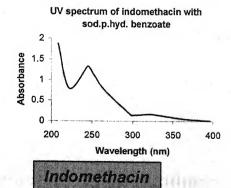


Fig. 4.1.6(II): UV spectrum of chlorzoxazone and indomethacin with hydrotropes

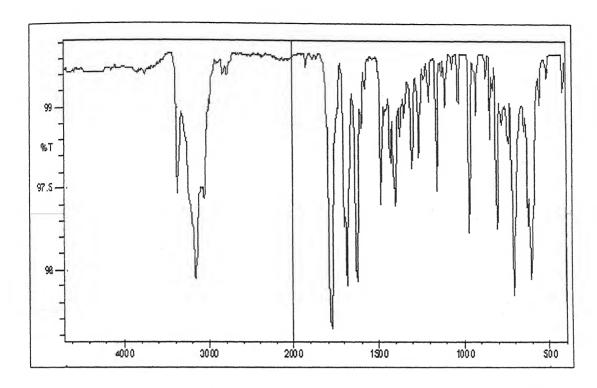


Fig. 4.1.26: FTIR spectrum of chlorzoxazone+nicotinamide (PM)

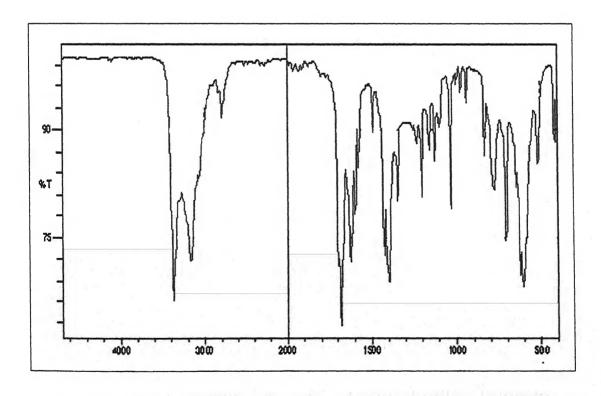


Fig. 4.1.27: FTIR spectrum of chlorzoxazone+nicotinamide (Solubilized)

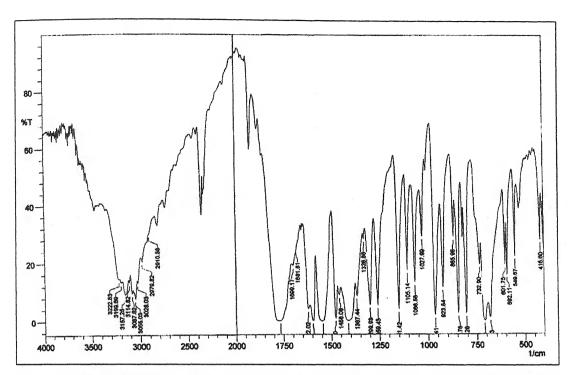


Fig. 4.1.28: FTIR spectrum of chlorzoxazone+sodium benzoate (PM)

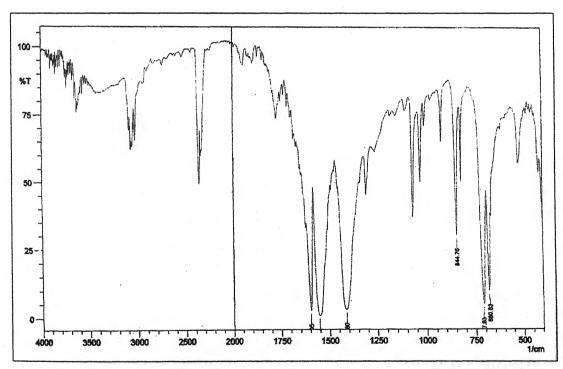


Fig. 4.1.29: FTIR spectrum of chlorzoxazone+sodium benzoate (Solubilized)

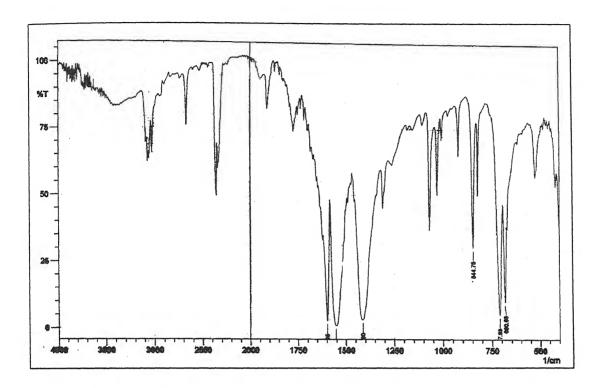


Fig. 4.1.30: FTIR spectrum of chlorzoxazone+sodium p-hydroxy benzoate (Solubilized)

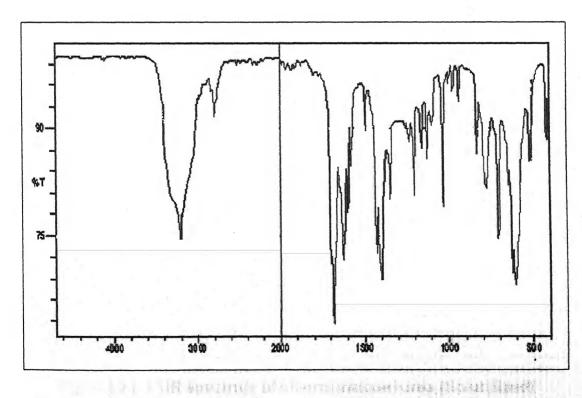


Fig. 4.1.31: FTIR spectrum of chlorzoxazone+resorcinol (Solubilized)

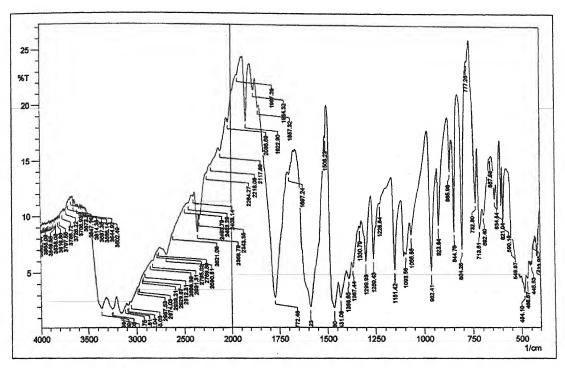


Fig. 4.1.32: FTIR spectrum of chlorzoxazone+urea (PM)

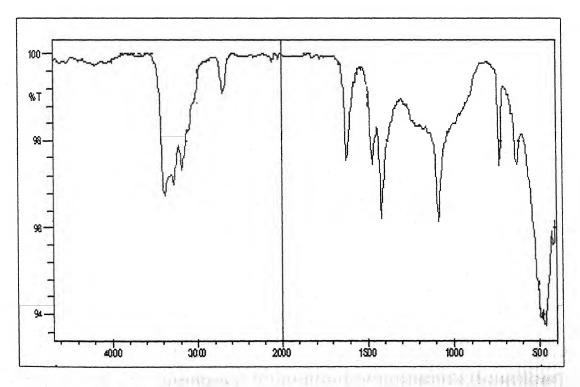


Fig. 4.1.33: FTIR spectrum of chlorzoxazone+urea (Solubilized)



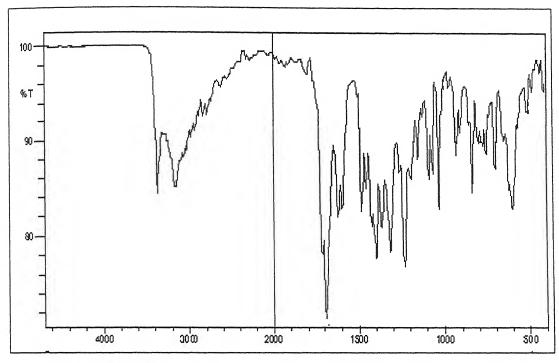


Fig. 4.1.34: FTIR spectrum of indomethacin+nicotinamide (PM)

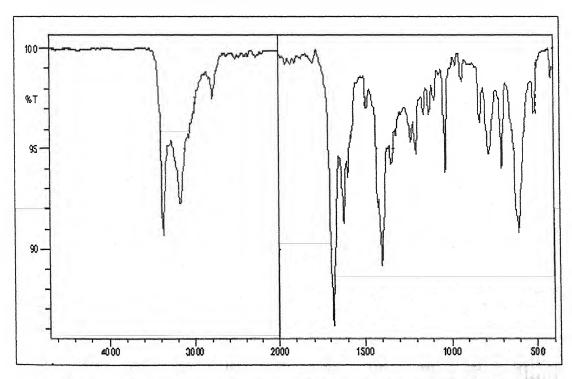


Fig. 4.1.35: FTIR spectrum of indomethacin+nicotinamide (Solubilized)

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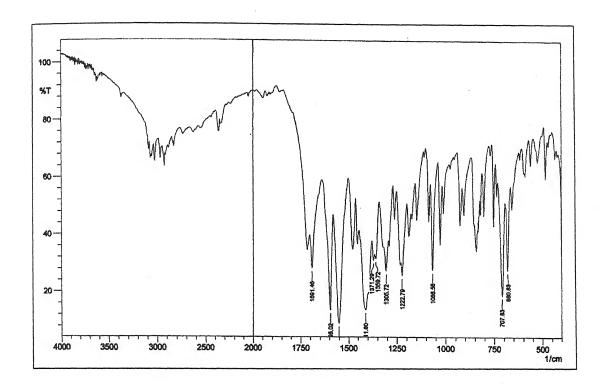


Fig. 4.1.36: FTIR spectrum of indomethacin+sodium benzoate (PM)

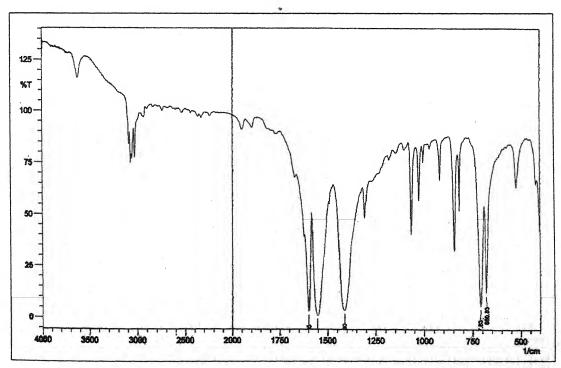


Fig. 4.1.37: FTIR spectrum of indomethacin+sodium benzoate (Solubilized)

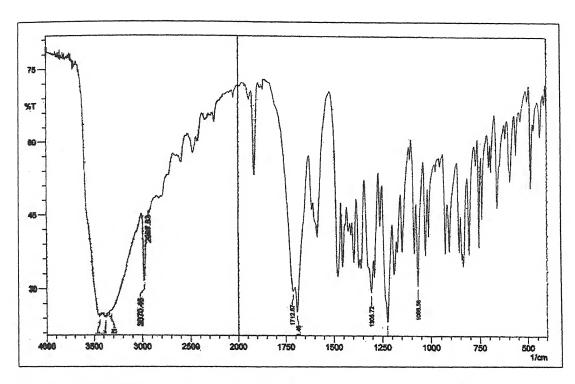


Fig. 4.1.38: FTIR spectrum of indomethacin+sodium p-hydroxy benzoate (PM)

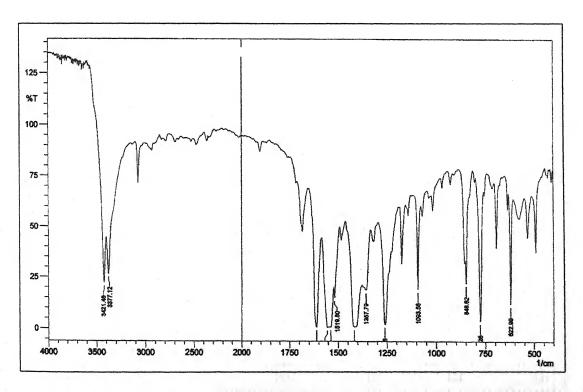


Fig. 4.1.39: FTIR spectrum of indomethacin+sodium p-hydroxy benzoate (Solubilized)

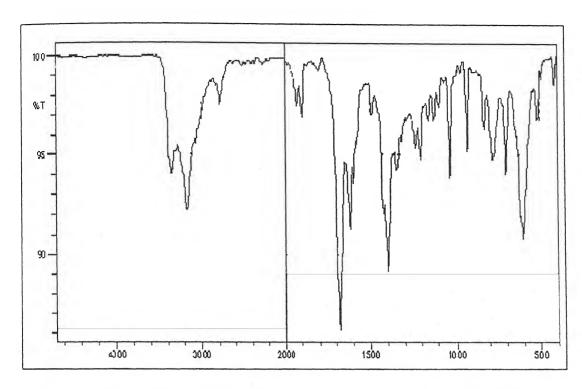


Fig. 4.1.40: FTIR spectrum of indomethacin+resorcinol (Solubilized)

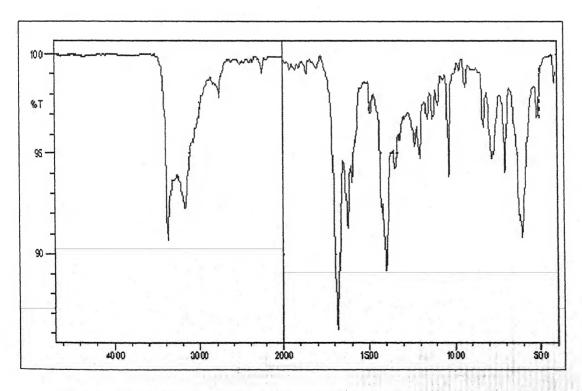


Fig. 4.1.41: FTIR spectrum of indomethacin+urea (Solubilized)

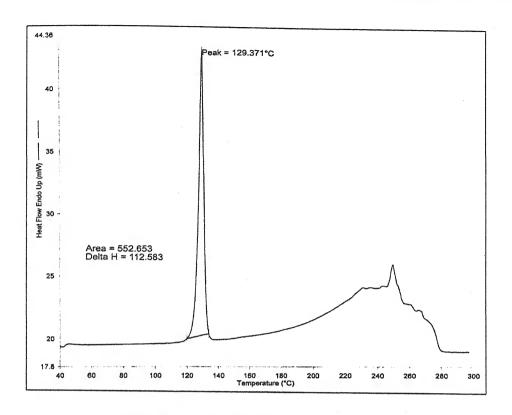


Fig. 4.1.42: DSC curve of nicotinamide

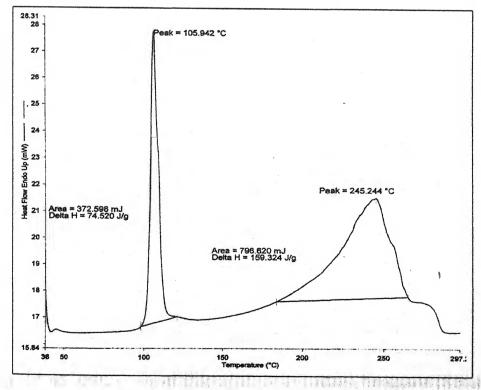


Fig. 4.1.43: DSC curve of chlorzoxazone+nicotinamide (PM)



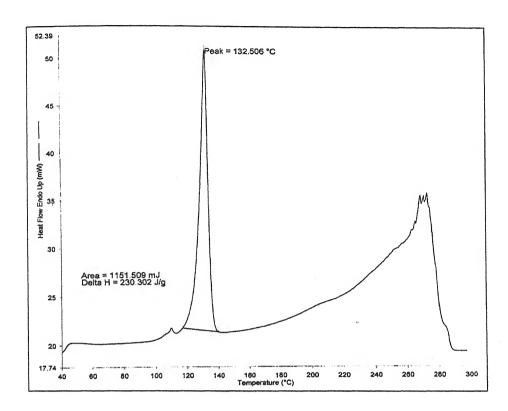


Fig. 4.1.44: DSC curve of chlorzoxazone+nicotinamide (Solubilized)

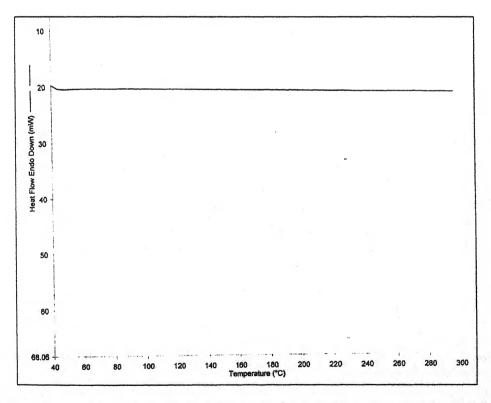


Fig. 4.1.45: DSC curve of chlorzoxazone+sodium benzoate (Solubilized)

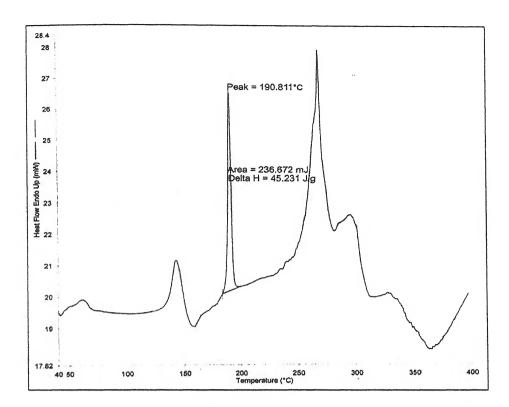


Fig. 4.1.46: DSC curve of chlorzoxazone+sodium p-hydroxy benzoate (PM)

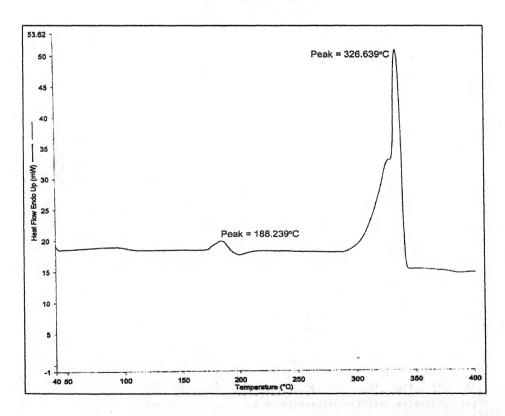


Fig. 4.1.47: DSC curve of chlorzoxazone+sodium p-hydroxy benzoate (Solubilized)

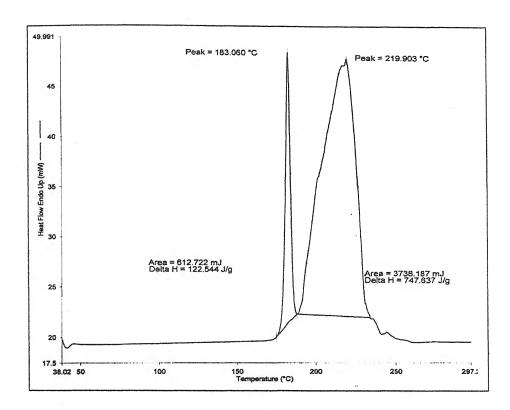


Fig. 4.1.48: DSC curve of chlorzoxazone+urea (Solubilized)

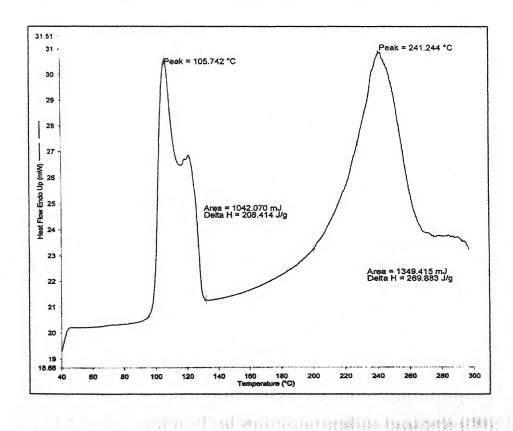


Fig. 4.1.49: DSC curve of indomethacin+nicotinamide (PM)

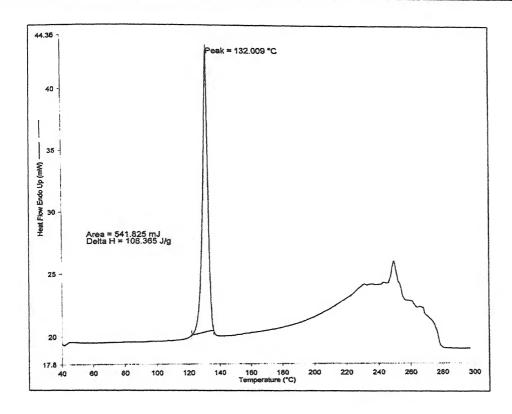


Fig. 4.1.50: DSC curve of indomethacin+nicotinamide (Solubilized)

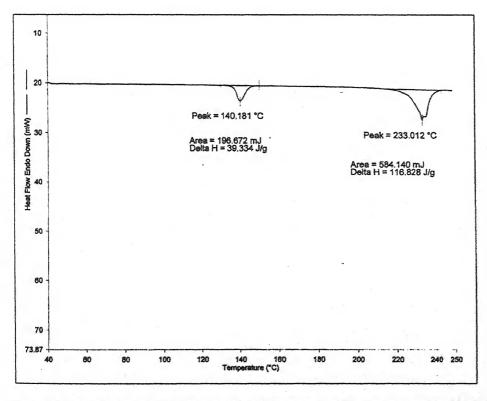


Fig. 4.1.51: DSC curve of indomethacin+sodium benzoate (PM) Marie College (17)

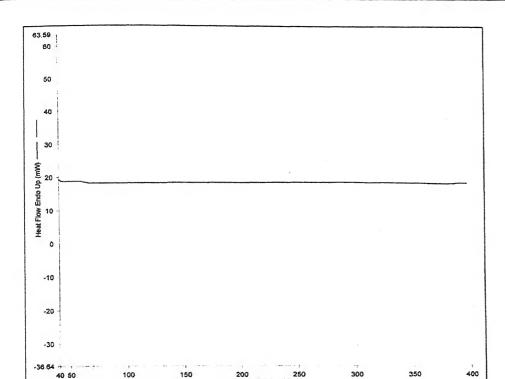


Fig. 4.1.52: DSC curve of indomethacin+sodium benzoate (Solubilized)

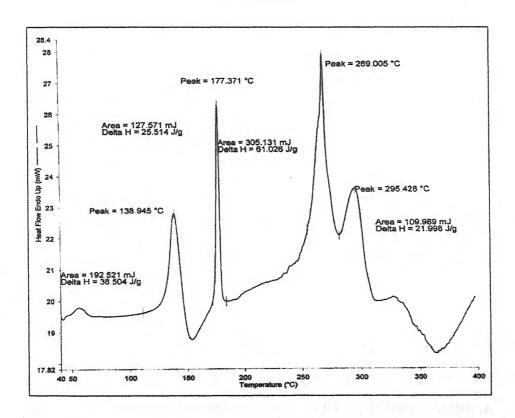


Fig. 4.1.53: DSC curve of indomethacin+sodium p-hydroxy benzoate (PM)

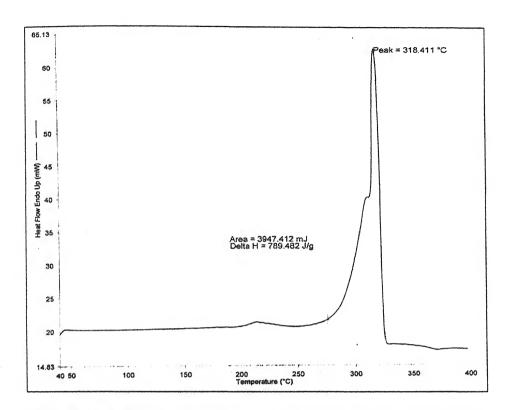


Fig. 4.1.54: DSC curve of indomethacin+sodium p-hydroxy benzoate (Solubilized)

4.1.5.5 X-ray powder diffraction

X-ray diffraction patterns of dried solubilized products and physical mixture of the each drug with various hydrotropes were obtained at room temperature (25°C) using a D-8 Advance, Bruker-AXS Diffractometer (Germany). Samples were exposed to Cu K α radiation at scanning rate 2°/min with step size 0.050°, step time 1.5 second over scanning range 3.000° to 120.000° of the diffraction angle 26; the generator was set to 40 kV and 30mA. X-ray diffraction patterns are shown in figure 4.1.55-4.1.64.

4.1.5.6 Microscopic studies

The drugs, Hydrotropes and their solubilized products were examined microscopically for their crystallinity and morphological characteristics and recorded by photomicrograph using Ezee capture camera. The photomicrographs are shown in plate 1-3.

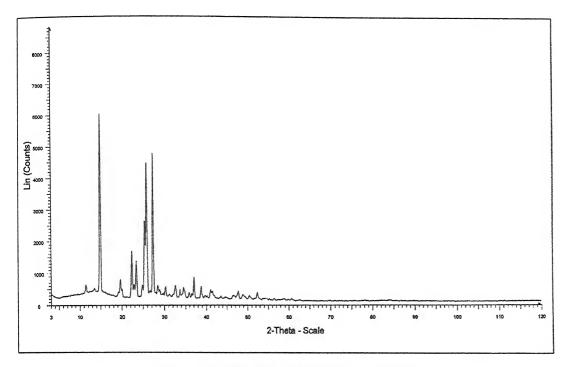


Fig. 4.1.55: XRPD of nicotinamide

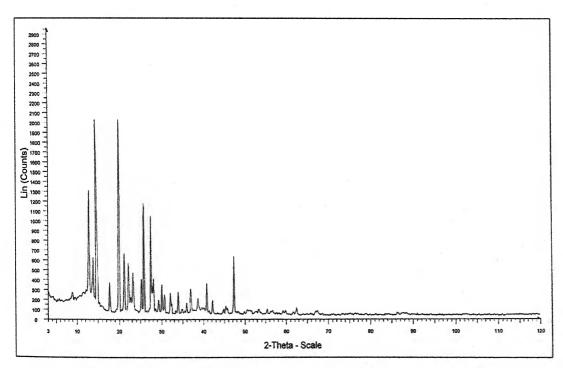


Fig. 4.1.56: XRPD of chlorzoxazone+nicotinamide (PM)



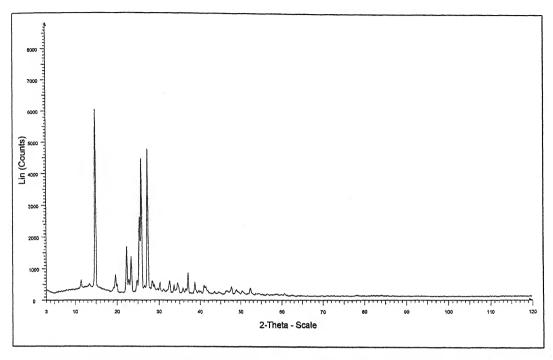


Fig. 4.1.57: XRPD of chlorzoxazone+nicotinamide (Solubilized)

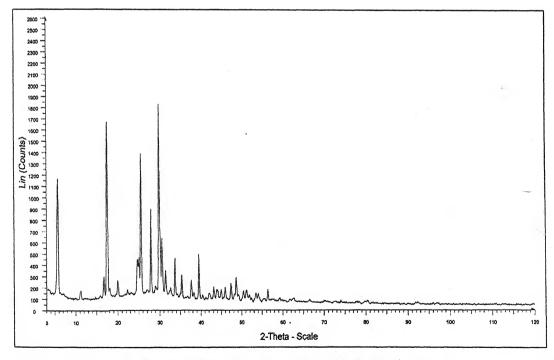


Fig. 4.1.58: XRPD of sodium p-hydroxy benzoate

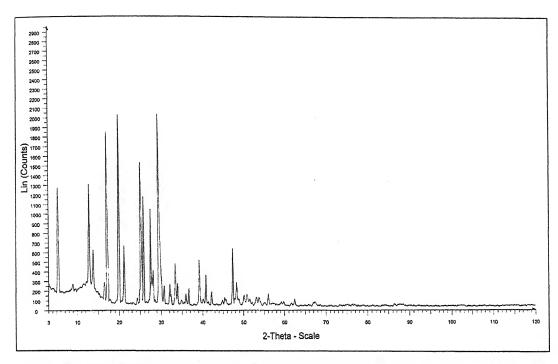


Fig. 4.1.59: XRPD of chlorzoxazone+sodium p-hydroxy benzoate (PM)

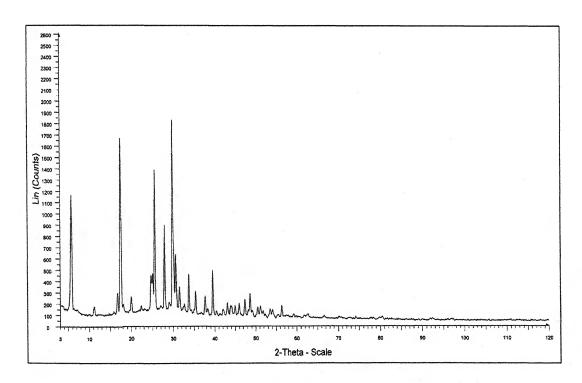


Fig. 4.1.60: XRPD of chlorzoxazone+sodium p-hydroxy benzoate (Solubilized)

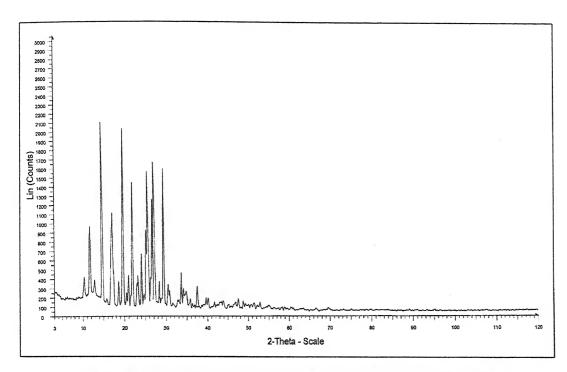


Fig. 4.1.61: XRPD of indomethacin+nicotinamide (PM)

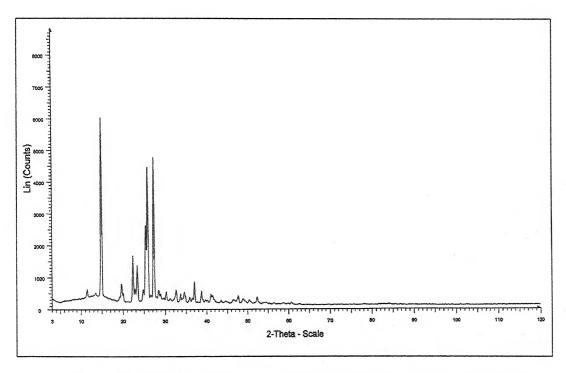


Fig. 4.1.62: XRPD of indomethacin+nicotinamide (Solubilized)

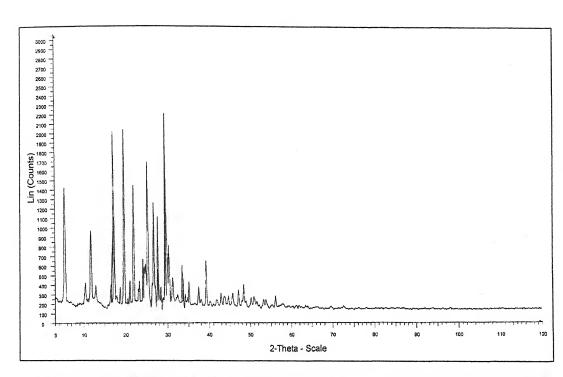


Fig. 4.1.63: XRPD of indomethacin+sodium p-hydroxy benzoate (PM)

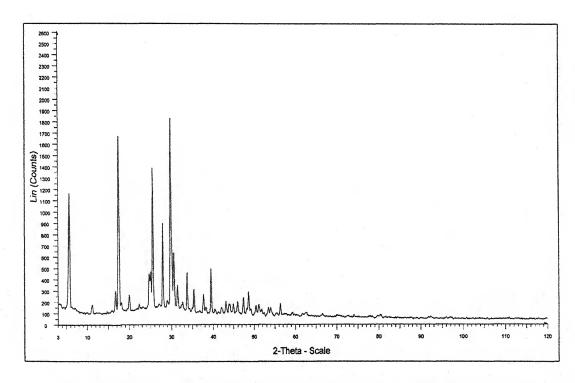


Fig. 4.1.64: XRPD of indomethacin+sodium p-hydroxy benzoate (Solubilized)



Nicotinamide (100X)



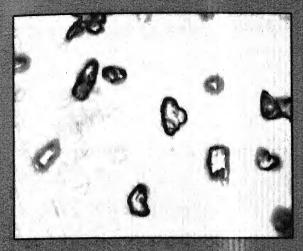


Sadium benzaste (400%)





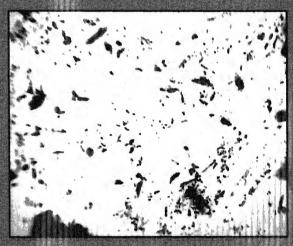
Plate 1: Photomicrograph of Hydrotropes



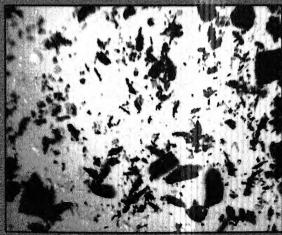
Chlorzoxazone sodium benzoate solubilized (100X)



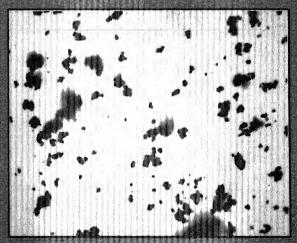
Chlorzoxazone resorcinol solubilized (100X)



Chlorzoxazone nicotinamide solubilized (100X)



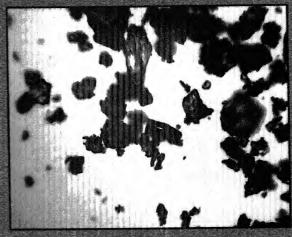
Chlorzoxazone sodium benzoate solubilized (100X)



Chlorzoxazone sodium p-hydroxy benzoste solubilized (100X)



Indomethacin sodium benzoate solubilized (100X)



Indomethacin resorcinol solubilized (100X)



Indomethacin nicotinamide solubilized (100X)



Indomethacin sodium benzoate solubilized (100X)



Indomethagin sodium p-hydroxy penzoate solubilized (100X)

Fig. 4.1.65: Chemical structure of drugs and hydrotropes

benzoate

Fig. 4.1.66: Interaction of Chlorzoxazone with Nicotinamide in aqueous solution

Fig. 4.1.67: Interaction of Chlorzoxazone with Sodium benzoate in aqueous solution

Fig. 4.1.68: Interaction of Chlorzoxazone with Sodium p-hydroxy benzoate in aqueous solution

Fig. 4.1.69: Interaction of Chlorzoxazone with Resorcinol in aqueous solution

Fig. 4.1.70: Interaction of Chlorzoxazone with Urea in aqueous solution

Fig. 4.1.71: Interaction of Indomethacin with Nicotinamide in aqueous solution

Fig. 4.1.72: Interaction of Indomethacin with Sodium Benzoate in aqueous solution

Fig. 4.1.73: Interaction of Indomethacin with Sodium p-hydroxy Benzoate in aqueous solution

Fig. 4.1.74: Interaction of Indomethacin with Resorcinol in aqueous solution

Fig. 4.1.75: Interaction of Indomethacin with Urea in aqueous solution

4.1.6 Mathematical Analysis of Phase Solubility Data 16

The interaction of the drugs with hydrotropes was studied using standard phase solubility method. The data were analyzed by assuming that both 1:1 and 1:2 complexes could be formed in accordance with the following relationships:

$$[S_W] + [L] = [SL]$$
(1)

$$[S_W] + [2L] = [SL_2]$$
(2)

Where $[S_W]$ is the equilibrium solubility of the drugs in the absence of hydrotropes, [L] is the molar concentration of the free hydrotrope, [SL] is the molar concentration of the 1:1 drug:hydrotrope complex, [2L] is the molar concentration of hydrotrope dimers, and $[SL_2]$ is the molar concentration of the 1:2 drug:hydrotrope complex.

 $K_{1:1}$ and $K_{1:2}$ stability constants are calculated using the following equations:

$$K_{1:1} = \frac{[SL]}{[S_W][L]}$$
 ...(3)

$$K_{1:2} = \frac{[SL_2]}{[S_W][L]^2}$$
 ...(4)

$$\frac{[S_T]-[S_W]}{[L_T]-2([S_T]-[S_W])} = \alpha + \beta\{[L_T] - 2([S_T])\} \qquad ...(5)$$

Where $[S_T]$ is the total molar concentration of the drug and $[L_T]$ is the total molar concentration of the ligand and where;

$$\alpha = \frac{K_{1:1} [S_W]}{1 - K_{1:1} [S_W]} ...(6)$$

$$\beta = \frac{K_{1:2} [S_W]}{(1 - K_{1:1} [S_W])^2} ...(7)$$

A plot of the left side of equation 5 versus $[L_T]$ - $2([S_T]-[S_W])$ gives a straight line with a slope equal to β and an intercept equal to α . The stability constants, $K_{1:1}$ and $K_{1:2}$, can be calculated from the intercept and slope, respectively.

4.1.7 Estimation of Thermodynamic Parameters for the Stability Constant

The free energy change ΔG is determined from the stability constant K by the expression:

$$\Delta G = -2.303RT \log K \qquad ...(8)$$

Where R is the gas constant (8.3143 J.mol⁻¹.K⁻¹) and T is the absolute temperature. The enthalpy change ΔH is estimated from the stability constant at several temperatures by the following relationship.

$$\log K = -\frac{\Delta H}{2.303R} \times \frac{1}{T} + \text{const.}$$
 ...(9)

A linear plot of log K against 1/T (a van't Hoff plot) yields ΔH from slope. The entropy change ΔS is related to ΔG and ΔH as:

$$\Delta S = \frac{\Delta H - \Delta G}{T} \qquad ...(10)$$

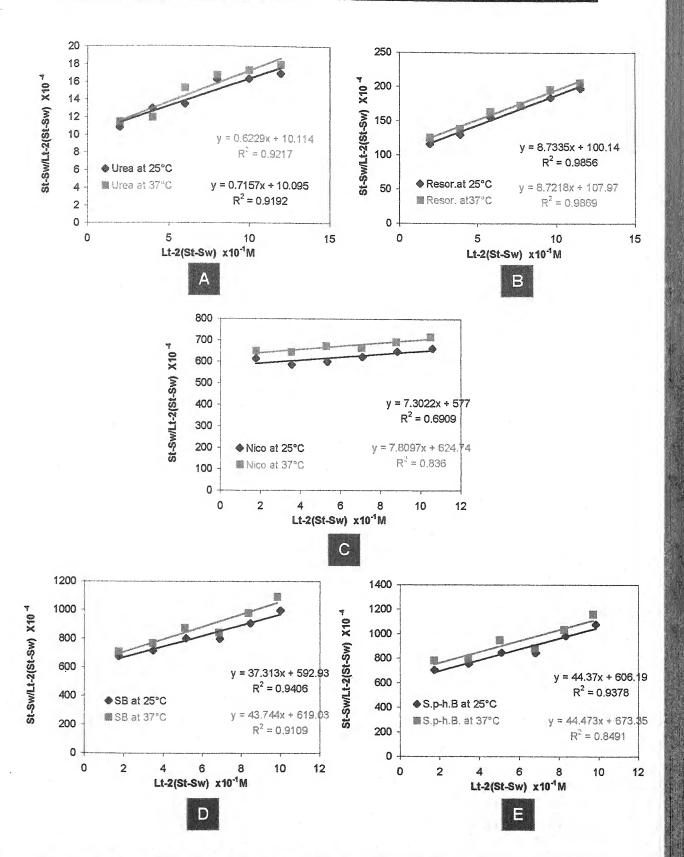


Fig. 4.1.76: Plots for determination of stability constants for chlorzoxazonehydrotrope (A) Urea (B) Resorcinol (C) Nicotinamide (D) Sodium Benzoate (E) Sodium p-Hydroxy Benzoate interactions

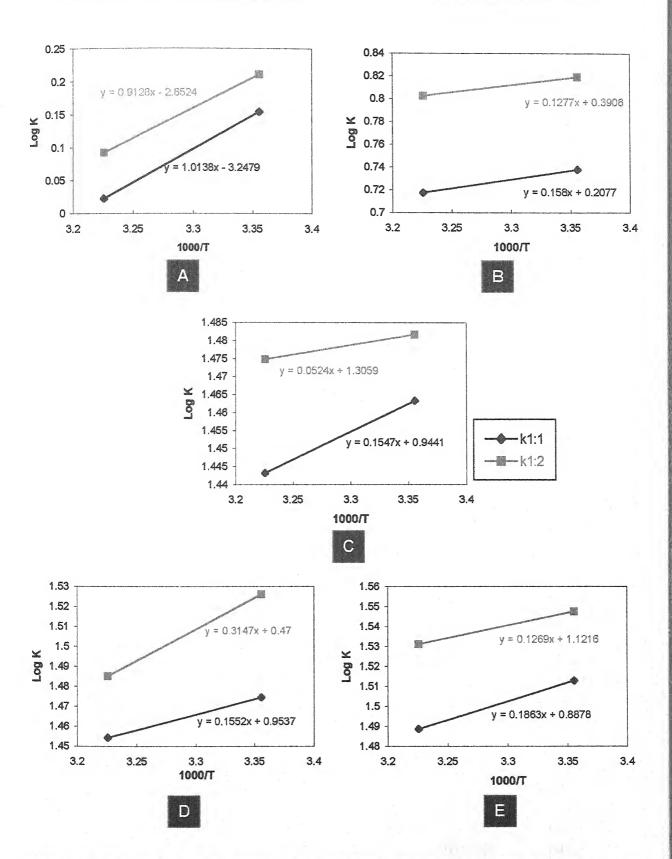


Fig. 4.1.77: Van't Hoff Plots of stability constants for chlorzoxazone-hydrotrope (A) Urea (B) Resorcinol (C) Nicotinamide (D) Sodium Benzoate (E) Sodium p-Hydroxy Benzoate interactions

Table 4.1.18: Stability constant for chlorzoxazone-hydrotrope interaction

S.No.	Hydrotrope	Temperature (°C)	Solubility in Water (mM)	K _{1:1} (M ⁻¹)	K _{1:2} (M ⁻²)
1	Nicotinamide	25±2	1.88	29.06	30.31
		37±2	2.05	27.75	29.84
2	Sodium benzoate	25±2	1.88	29.82	33.57
		37±2	2.05	28.46	30.56
3	Sodium p-hydroxy benzoate	25±2	1.88	32.57	35.28
		37±2	2.05	30.80	33.96
4	Resorcinol	25±2	1.88	5.47	6.59
		37±2	2.05	5.22	6.35
5	Urea	25±2	1.88	1.43	1.62
		37±2	2.05	1.05	1.24

Table 4.1.19: Thermodynamic parameter for chlorzoxazone hydrotrope interaction

S.No.	Hydrotrope	Stability Constant	Temp. (°C)	∆G (kJ.mol ⁻¹)	ΔH (kJ.mol ⁻¹)	ΔS (kJ.mol ⁻¹ .K ⁻¹)
1.	Nicotinamide	K _{1:1}	25±2	-8.35	-2962.17	-9.91
		131:1	37±2	-8.23	-2962.17	-9.53
'.		K _{1:2}	25±2	-8.45	-1003.35	-3.34
		1\1:2	37±2	-8.41	-1003.35	-3.21
	Sodium benzoate	K _{1:1}	25±2	-8.41	-2971.74	-9.94
2.		181:1	37±2	-8.30	-2971.74	-9.56
<i>-</i> .		K _{1:2}	25±2	-8.71	-6025.82	-20.19
			37±2	-8.47	-6025.82	-19.41
	Sodium p- hydroxy benzoate	K _{1:1}	25±2	-8.63	-3567.24	-11.94
3.			37±2	-8.49	-3567.24	-11.48
0.		K _{1:2}	25±2	-8.83	-2429.86	-8.12
			37±2	-8.73	-2429.86	-7.81
	Resorcinol	K _{1:1}	25±2	-4.21	-3025.36	-10.14
4.			37±2	-4.09	-3025.36	-9.75
		K _{1:2}	25±2	-4.67	-2445.18	-8.19
			37±2	-4.58	-2445.18	-7.87
	Urea	K _{1:1}	25±2	-0.88	-19412.07	-65.14
5.			37±2	-0.13	-19412.07	-62.62
J.		K _{1:2}	25±2	-1.20	-17478.14	-58.65
			37±2	-0.52	-17478.14	-56.38



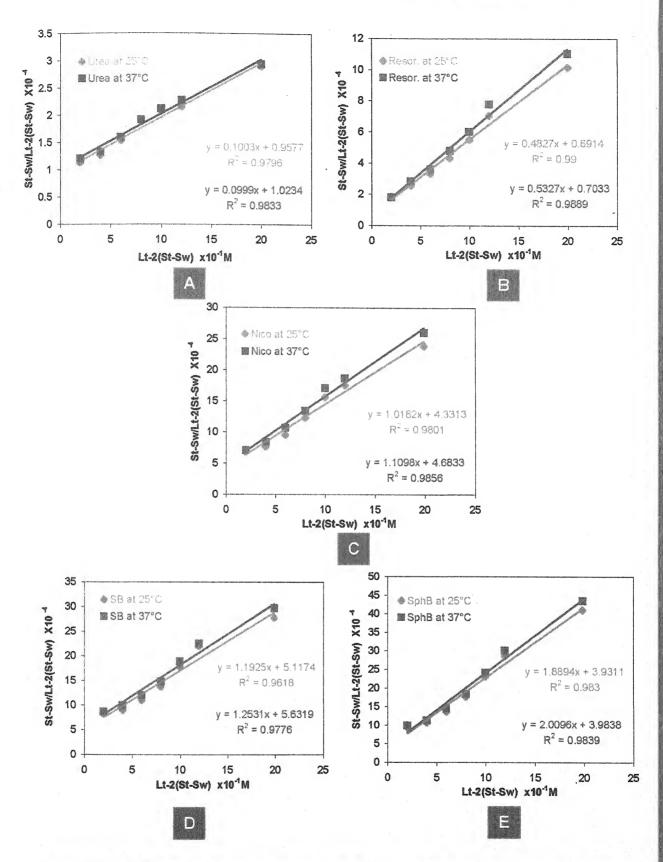


Fig. 4.1.78: Plots for determination of stability constants for indomethacinhydrotrope (A) Urea (B) Resorcinol (C) Nicotinamide (D) Sodium Benzoate (E) Sodium p-Hydroxy Benzoate interactions

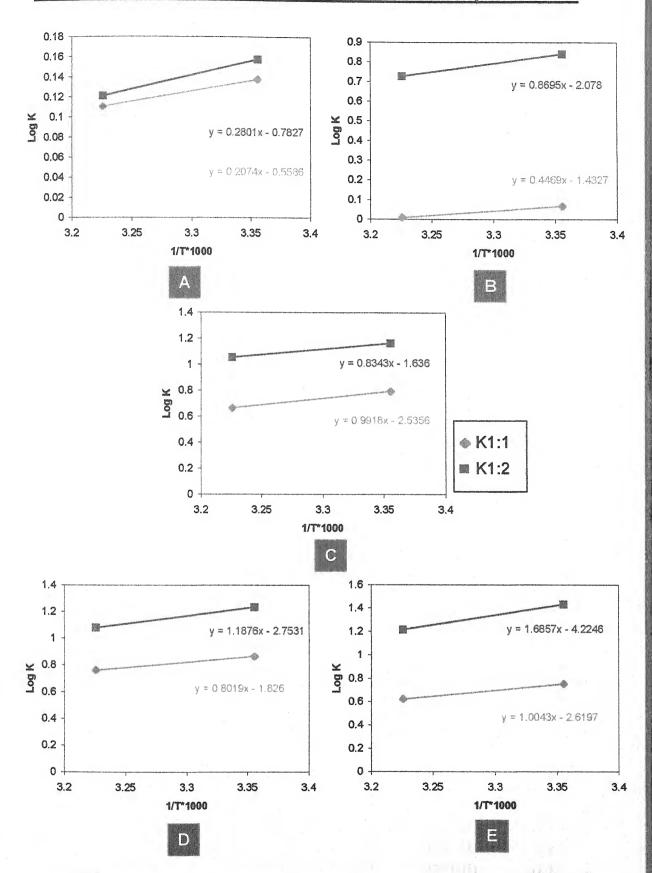


Fig. 4.1.79: Van't Hoff Plots of stability constants for indomethacin-hydrotrope
(A) Urea (B) Resorcinol (C) Nicotinamide (D) Sodium Benzoate
(E) Sodium p-Hydroxy Benzoate interactions

Table 4.1.20: Stability constant for indomethacin hydrotrope interaction

S.No.	Hydrotrope	Temperature (°C)	Solubility in Water (mM)	K _{1:1} (M ⁻¹)	K _{1:2} (M ⁻²)
1	Nicotinamide	25±2	0.07	6.20	14.58
	Modulamac	37±2	0.079	5.90	13.97
2	Sodium benzoate	25±2	0.07	7.33	17.07
	Oddidiii belizedie	37±2	0.079	7.09	15.77
3	Sodium p-hydroxy benzoate	25±2	0.07	5.63	27.05
3		37±2	0.079	5.02	25.30
4	Resorcinol	25±2	0.07	1.17	6.91
7		37±2	0.079	1.02	6.71
5	Urea	25±2	0.07	1.37	1.44
3	Olea	37±2	0.079	1.29	1.32

Table 4.1.21: Thermodynamic parameter for indomethacin hydrotrope interaction

S.No.	Hydrotrope	Stability Constant	Temp. (°C)	∆G (kJ.mol ⁻¹)	ΔH (kJ.mol ⁻¹)	ΔS (kJ.mol ⁻¹ .K ⁻¹)
		K _{1:1}	25±2	-4.52	-18990.82	-63.71
1.	Nicotinamide	1\1:1	37±2	-4.40	-18990.82	-61.25
1.	Nicotinamiae	K _{1:2}	25±2	-6.64	-15975.04	-53.59
		171:2	37±2	-6.53	-15975.04	-51.51
		K _{1:1}	25±2	-4.93	-15354.65	-51.51
2.	Sodium	18414	37±2	-4.85	-15354.65	-49.52
۵.	benzoate	K _{1:2}	25±2	-7.03	-22739.97	-76.29
		174:2	37±2	-6.83	-22739.97	-73.33
		K _{1:1}	25±2	-4.28	-19230.17	-64.52
3.	Sodium p- hydroxy		37±2	-4.00	-19230.17	-62.02
0.	benzoate	K _{1:2}	25±2	-8.17	-32277.50	-108.29
****			37±2	-8.00	-32277.50	-104.10
	Resorcinol	K _{1:1}	25±2	-0.38	-8557.17	-28.71
4.			37±2	-0.05	-8557.17	-27.60
7.		K _{1:2}	25±2	-4.79	-16649.04	-55.85
			37±2	-4.72	-16649.04	-53.69
5.		K _{1:1}	25±2	-0.78	-3971.26	-13.32
	Urea		37±2	-0.63	-3971.26	-12.81
	Olca	K _{1:2}	25±2	-0.90	-5363.31	-17.99
			37±2	-0.69	-5363.31	-17.30

4.1.8 Results and Discussion

The solubility of chlorzoxazone and indomethacin was evaluated in five hydrotropes namely urea, resorcinol, nicotinamide, sodium benzoate and sodium para-hydroxy benzoate in concentration of 0.2 M to 1.2 or 2.0M aqueous solution. The solubility of both the drugs improved several folds in higher hydrotropes concentration. The solubility of chlorzoxazone in 1.2 M urea, resorcinol, nicotinamide, sodium benzoate and sodium para-hydroxy benzoate was found to be 0.662±0.045, 4.18±0.166, 12.225±0.529, 17.215±0.812 and 18.355±0.618 mg/ml at 25°C respectively, which was 2.080, 13.44, 38.405, 54.081 and 57.662 fold that of aqueous solubility of chlorzoxazone (Table 4.1.2-4.1.6). The solubilizing power of different hydrotropes for chlorzoxazone could be ranked as sodium pera-hydroxy benzoate > sodium benzoate > Nicotinamide > Resorcinol > Urea.

Similarly solubility of indomethacin in 2.0 M urea, resorcinol, nicotinamide, sodium benzoate and sodium pera hydroxy benzoate was found to be 0.232±0.027, 0.750±0.103, 1.725±0.171, 2.000±0.227, 2.934±0.435 mg/ml respectively at 25°C, which was 9.291, 30.043, 69.065, 80.076, 117.499 fold that of aqueous solubility of indomethacin (Table 4.1.7-4.1.11). The solubilizing power of different hydrotropes for indomethcin could be ranked as sodium para-hydroxy benzoate > sodium benzoate > Nicotinamide > Resorcinol > Urea.

The observation of phase solubility diagram of chlorzoxazone and indomethacin in aqueous solution of the five hydrotropes from concentration of 0.2 M to 1.2 (for chlorzoxazone) or 2.0 M (for indomethacin) revealed that the drugs were more soluble at higher hydrotrope concentration. The phase solubility diagram was Ap type. This shows that increase in total solubility of drug was not linear function of hydrotropic concentration. This positive deviation from linearity is due to formation of water-soluble complexes of higher order between drugs and solubilizer, typical of hydrotropism. The Solubilization seemed to be continuous phenomenon as solubility increased even at very low concentrations of ligand. The critical hydrotropic concentration, at which hydrotropic action begins, therefore was not determined.

It has been shown that higher order complexes are formed as a result of different type of interactions. One type of interaction may involve formation of dimmers or aggregates containing two or more molecules of ligand but high order complexes also formed via a stepwise interaction involving the substrate and two molecules of ligand A characteristic that many hydrotropic agents share the ability to self-associate in aqueous solution at hydrotropic concentration. The observed decrease in solubility at higher temperature is due to lower self-association of hydrotrope at higher temperature.

Mukerjee¹⁹ proposed that the molecule having exposed organic groups that are not protected by polar groups on more than one side, show some degree of hydrophobicity and associated, in presence of water. The phenomenon is termed as hydrophobic bonding. This aggregation may be an inter and/or intramolecular, and depends on the concentration of hydrophobic moieties present in the system. The hydrotropes are essentially planar structure and allow a staking type of association, where in each monomer can lie flat on the top of another monomer. Interaction of solutes with these aggregates is significant contributor to the dramatic increase in solubility by hydrotropes.

The stability constant of complex formation $K_{1:1}$ and $K_{1:2}$ were determined for drug hydrotrope in 1:1 and 1:2 ratio respectively by non-linear least square regression analysis of the solubility data (Table 4.1.18 and 4.1.20).

The result indicate that increasing the hydrotrope quantity in the system favors formation of high order complexes as the value of $K_{1:2}$ is greater than $K_{1:1}$. For drug complexes, K values depend strongly on the ligand employed and temperature of system and the greater the stability constant the greater the solubility. The lower values of $K_{1:2}$ with increasing temperature indicate decrease in ligand hydrotropic ability. These findings suggest role of self-association in hydrotropy that decreases with increasing temperature. A study by Kopecky et al.²⁰ has documented lesser extent of self-association of hydrotropes.

To explain the mechanism of solubilization of chlorzoxazone and indomethacin in presence of structurally different hydrotropes, it is necessary

to have basic knowledge of chemical structure of hydrotropes and different centres with different electronegativity in drug and hydrotrope molecule (Fig. 4.1.65).

The higher solubility of indomethacin in presence of one hydrotrope over other can be explained on the basis of Poochikian and Gradock's explanation²¹. The hydrotropes selected for the present study (nicotinamide, Resorcinol, sodium benzoate and sodium p-hydroxy benzoate) possess a hydrophobic center having the parent benzene nucleus which can interact due to a large surface area and a mobile electron cloud known as an aromatic sextet. Thus these sites are available for non-bonded and Vander Wall's interaction with water and indomethacin. The molecules of water join to form cluster together. For solubilization, the ionized hydrotropes break this association and use the ion dipoles of water for salvation.

The increasing hydrotrope concentration results in unassociated form of water to make cluster of the hydrotrope by hydrogen bonding and non-bonding interactions at the various centers of drug molecule. Thus charge delocalization along with an increase in ™cloud area on hydrotropic molecule would account partially for difference in apparent drug solubility in presence of various hydrotropes²¹.

Nicotinamide, a vitamin B_3 , is well known as a hydrotropic agent, and its most commonly proposed solubilization mechanism is complexation $^{22-29}$. The formation of complex species between nicotinamide and certain heteroaromatic drug molecules has been shown by molecular orbital calculation to occur via a is π -donor π -acceptor mechanism $^{26-28}$. Using nicotinamide and its related compounds, Rasool et~al. also showed that the aromaticity (π -system) of the pyridine ring which might promote the stacking of molecules through its planarity, was an important factor in complexation because the aromatic amide ligands enhanced the aqueous solubilities of the test drugs to a greater extant than the aliphatic amide ligands. On the other hand, Kenley et~al. revealed that the hydrophobicity of ligands including nicotinamide was a general determinant of water-soluble complex formation, and donor-acceptor interactions did not control complex formation for the substrate-ligand combinations they considered.

The interaction of sodium benzoate with chlorzoxazone give solubility enhancement ratio about 54 in 1.2 m sodium benzoate solution. The intramolecular hydrogen bonding and charge transfer phenomenon seem to be contribution in solubility enhancement. Also chlorzoxazone is more soluble in higher pH and sodium benzoate increase the pH of the solution. So increase in solubility due to greater percentage of ionized chlorzoxazone.

The interaction of sodium benzoate with indomethacin can be explained as the interaction of a weak acid and with a salt of strong alkali. Even though the solubility enhancement ratio is about 80 times in 2.0 M sodium benzoate solution.

Sodium p-hydroxy benzoate increases the solubility of chlorzoxazone 57.7 fold in 1.2 M solution and of indomethacin to 117.5 fold in concentration of 2.0 M solution. Thus sodium p-hydroxy benzoate gives highest solubility enhancement among the five hydrotrope for both the drugs. This may be due to increase in pH of solution due strong alkali base and both drugs are soluble al higher pH.

Resorcinol having two phenolic group contributes to hydrogen bonding contributes to hydrophilicity and aromatic ring may contributes to planner type of stacking compexation with both the drugs.

To examine the solubilizing effect of aliphatic amide in water the solubility of both the drugs was also determined in 0.2M to 1.2 or 2.0 molar solution of Urea. Figure 4.1.7 and 4.1.14 are graphs of chlorzoxazone and indomethacin solubility as a function of increasing urea concentration in aqueous solution. From that graph it can be concluded that urea does indeed hydrotropically solubilize chlorzoxazone and indomethacin. It is interesting to note that significant increase in solubility of chlorzoxazone or indomethacin was not evident until the concentration of urea reaches approximately 0.8M.

The plots of specific gravity versus hydrotrope concentration showed a negative deviation (Fig. 4.1.21) that indicates an increase in partial molal volume upon aggregation, and this increase in volume may be due to expansion of the hydrocarbon portion of the molecule or its partial removal from the high compressive force of water¹¹. The positive deviation in the

viscosity plots (Fig. 4.1.22) indicates that aggregate formation is associated with an increase in viscosity of hydrotrope concentration, which is in agreement with the self-association of phenolic compounds³⁰. The surface tension plots (Fig. 4.1.23) showed a moderate decrease in surface tension on increasing the hydrotrope concentration as hydrotropes are not surface active agents^{31,32}. The plots of refractive index versus hydrotrope concentration (Fig. 4.1.24) showed negative deviation. The deviation from linearity in specific conductance plots (Fig. 4.1.25) is strongly indicative of molecular aggregation³³. It was revealed from different studies that at lower hydrotrope concentration, weak ionic interactions while at higher hydrotrope concentration, the molecular aggregation seems to be the possible mechanism of hydrotropic solubilization^{1,2,31,34}.

To explain the increase in solubility of chlorzoxazone and indomethacin at lower hydrotrope concentration UV spectral studies of drug in different hydrotrope were conducted. Chlorzoxazone gives single λ_{max} at 280 nm in distilled water. Except nicotinamide in all other hydrotopes solution, no new peaks appeared, although a very minor shift of 0.5 nm in parent peak of chlorzoxazone was observed (Table 4.1.2). However with nicotinamide a bathochromic shift of 10.5 nm (280 to 269.5) was occurred. Similarly a shift in λ_{max} of nicotinamide 261.5 to 263 nm occurred. Shift of this type often associated with $\pi\text{--}\pi$ complexation. However, $\pi\text{--}\pi$ complexation requires a π electron donor and acceptor, π - π complexation would not be expected to occur between nicotinamide and chlorzoxazone because both are $\boldsymbol{\pi}$ electron acceptor. Small changes in peak position and absorbance like those seen here are also observed when the solvent medium in which the compound is analyzed is changed. This prompted to consider the possibility that nicrotinamide was affecting change in aqueous solubility of chlorzoxazone not by interacting with chlorzoxazone but by changing the nature of water as a solvent.

Similarly indomethacin gives two absorption maxima (λ_{max} 265 and 319.5 nm) in distilled water. The UV spectra of indomethacin in different hydrotrope solution have no significant change in λ_{max} of indomethacin at

319.5 nm as shown in table 4.1.1. However, the λ_{max} at 265 nm was masked in nicotinamide solution by λ_{max} of nicotinamide i.e. 261.5 nm in sodium p-hydroxy bezoate i.e. 246.5 nm and in resorcinol solution by the λ_{max} of resorcinol i.e. 272.5 nm.

The above finding suggests that increase in solubility of chlorzoxazone or indomethacin in different hydrotrope solutions is not due to any complex formation between drug and hydrotrope molecules, because the complex formation can be evidenced by formation of new chromophores (by appearance of new peak) or merging of two peaks to generate a common peak. But very slight shift in λ_{max} (±0.5 nm) might be due to minor possible effect of hydrotrope molecules on the electronic configuration of drug (chlorzoxazone/indomethacin) molecule. Thus the hydrotropic agent exert their solubilizing effect by changing the nature of the solvent, specially by altering the solvents ability to participate in structure formation via intermolecular hydrogen bonding.

Study of FTIR spectrum of chlorzoxazone, nicotinamide and their solubilized product revealed that through hydrogen bonding the carbonyl stretch of chlorzoxazone occurs at lower wave number than the carbonyl stretch of pure compound i.e. from 1770.53 to 1690 (Fig. 4.1.26-4.1.27). So it is ample proof that nicotinamide formed hydrogen bond with the oxygen of the carbonyl group of the drug and lowered the wave number of its, carbonyl stretch. Hence it imparted aqueous solubility in it, through various hydrogen bonding centres on hetro atoms with non-bonded electron pairs on them. These interactions are depicted in figure 4.1.66.

The observation of FTIR spectrum of chlorzoxazone, sodium benzoate and their solubilized product showed that due to ion dipole bond formation between sodium ion and carbonyl group of chlorzoxazone, the carbonyl group stretch wave number reduced from 1770.53 to 1596.95 (Fig. 4.1.28-4.1.29), further carbonyl group of sodium benzoate forms hydrogen bonding with H-N group of chlorzoxazone. London forces also act between non-polar parts of chlorzoxazone and sodium benzoate. These interactions thus imparting

aqueous solubility to chlorzoxazone in sodium benzoate solution. The possible interactions are shown in figure 4.1.64.

In case of sodium p-hydroxy benzoate, similar to sodium benzoate ion dipole bond formation between sodium ion and carbonyl group of chlorzoxazone was evident from carbonyl group stretch shift from 1770.53 to 1596.95 (Fig. 4.1.30). An additional hydroxyl group in sodium p-hydroxy benzoate than sodium benzoate imparted hydrogen bending with carbonyl group of another molecule of sodium p-hydroxy benzoate and also with carbonyl group of chlorzoxazone. Thus the aqueous solubility of chlorzoxazone is greater in sodium p-hydroxy benzoate the sodium benzoate. These interaction were visualized in figure 4.1.68.

In resorcinol solution the two hydroxyl groups form hydrogen bonding with various centers on chlorzoxazone molecule and forming a polar sheath around the chlorzoxazone molecule thus enhancing its aqueous solubility further weak wander wall and London forces also act between non-polar parts of chlorzoxazone and resorcinol (Fig. 4.1.69). These types of interactions imparting aqueous solubility to chlorzoxazone in resorcinol solution. Hydrogen bonding with carbonyl group of chlorzoxazone was evident from carbonyl stretch shift from 1770.53 to 1691 as shown by the FTIR spectrum (Fig. 4.1.31) of chlorzoxazone, resorcinol and their solubilized products.

Amide group of urea forms hydrogen bonding at various –ve centers of chlorzoxazone molecule and forming a polar sheath around chlorzoxazone molecule hence imparting it water solubility (Fig. 4.1.32-4.1.33). The possible interactions are shown in figure 4.1.70.

The FTIR spectrum of indomethacin and its solubilized product with nicotinamide revealed that through hydrogen bonding the carbonyl stretch of indomethacin molecule occurs at lower wave number (1680.23) than the two carbonyl stretches of pure indomethacin i.e. 1712.67 and 1619.46 (Fig. 4.1.34-4.1.35). This proves that nicotinamide formed hydrogen bonding with oxygen of the both carbonyl group of the drug and lowered the wave number of its carbonyl stretch. This way nicotinamide imparted aqueous solubility in it through various hydrogen bonding centers on hetro atoms with non-bonded

electron pair on it. Further there is possibility of London forces acting between the non-polar parts of both the molecule as shown in figure 4.1.71.

Study of FTIR spectrum of indomethacin, sodium benzoate and their solubilized product revealed that due ion dipole bonding with sodium ion the two carbonyl stretch of indomethacin shifted to lower wave number than the carbonyl stretch of pure compound i.e. 1712.67 and 1691.46 to 1596.95 (Fig. 4.1.36-4.1.37). This proves that sodium ion from sodium benzoate formed ion dipole bond with carbonyl group of indomethacin. Further London forces between aromatic rings (non-polar parts) and hydrogen bonding between polar parts also play role in imparting aqueous solubility enhancement of indomethacin by sodium benzoate. These interactions are shown in figure 4.1.72.

In case of indomethacin with sodium p-hydroxy benzoate hydrogen bonding between hydroxyl group of sodium p-hydroxy benzoate and carbonyl group of indomethacin and ion dipole interaction between Sodium ion and indomethacin seems to play major role. FTIR spectrum (Fig. 4.1.38-4.1.39) shown two carbonyl groups peaks of indomethacin shifted from 1712.67 and 1691.46 to 1596.95 due to interaction with sodium ion of sodium p-hydroxy benzoate. The spectrum also showed hydroxyl group stretch of pure sodium p-hydroxy benzoate shifted from 3415-3326 peaks to 1552. The figure 4.1.73 shows various centers of hydrogen bonding, ion dipole interaction and London forces of interaction.

The study of FTIR spectrum (Fig. 4.1.40) of indomethacin, resorcinol and their solubilized product revealed that resorcinol forms hydrogen bonding though its two hydroxyl group to various centers at indomethacin thus covering it with polar sheath and imparting it aqueous solubility as shown in figure 4.1.74.

Hydrogen bonding between amide group of urea and various negative centers of indomethacin molecule (Fig. 4.1.75) seems to impart aqueous solubility to indomethacin (Fig. 4.1.41).

A comparison among the endothermic transitions of chlorzoxazone, the pure hydrotropes their physical mixture and solubilized product of

chlorzoxazone in hydrotrope showed that thermograms of solubilized products have shifted peak temperature.

Physical mixture of chlorzoxazone and nicotinamide (Fig. 4.1.43) showed a sharp endothermic peak at 105.952°C attributed to melting and a broad endotherm at 245.244°C attributed to its decomposition instead of a endothermic peak of 130°C of pure nicotinamide (Fig. 4.1.42) and 194.399 of pure chlorzoxazone (Fig. 3.9), while their solubilized product showed a sharp endothermic peak at 132.506°C of melting and a broad endothermic above 200°C attributed to decomposition. The solubilized product had no evidence of a strong chlorzoxazone response at 194.399°C (Fig. 4.1.44). These finding suggests that either complexation have occurred or crystalline nature of chlorzoxazone has been changed and it converted into amorphous form after drying in nicotinamide solution.

Slubilized product of chlorzoxazone with sodium benzoate (Fig. 4.1.45) showed no endothermic transition upto 400°C suggesting amorphous form or possibility of complexation between chlorzoxazone and sodium benzoate.

Solubilized product of chlorzoxazone in sodium p-hydroxy benzoate (Fig. 4.1.47) was dissimilar to their physical mixture (Fig. 4.1.46) showed no endothermic peak attributed to melting of chlorzoxazone, thus suggesting possibility of complexation and/or alteration of crystal habit of chlorzoxazone.

Similarly a comparison among the endothermic transitions of indomethacin, pure hydrotropes, physical mixture of indomethacin and hydrotrope and their solubilized product showed that the thermograms of solubilized product of indomethacin with various hydrotropes have shifted peak temperatures as a evidence of either complex formation or change into crystalline structure of indomethacin.

Solubilized product of indomethacin in nicotinamide (Fig. 4.1.50) showed sharp peak at 132.009°C, which was attributed to melting of nicotinamide and a broad endotherm at about 249°C attributed to decomposition. No endothermic peak attributed to melting of indomethacin was observed.

Solubilized product of indomethacin in sodium benzoate (Fig. 4.1.52) showed no peak correspond to thermal transition up to 400°C. Physical mixture of indomethacin with sodium p-hydroxy benzoate (Fig. 4.1.53) showed various weak endothermic transition peaks i.e. 139°C, 177°C, 269°C and 295°C, while its solubilized product (Fig. 4.1.54) showed only on broad endotherm at 318°C.

Remarkable difference between solubilized product and the physical mixture of drug and hydrotrope was found in X-ray powder diffractometry. The diffraction pattern of physical mixture was simply the sum of those of components. While in solubilized product characteristic peaks of drug (chlorzoxazone or indomethacin) found to decrease intensity or disappeared (Fig. 4.1.55-4.1.64), suggesting drug precipitate as amorphous powder in hydrotrope solution. These results confirm the findings of DSC.

Thermodynamic parameters for 1:1 and 1:2 complex formation were estimated from van't Hoff plots of the temperature dependence of stability constants (Fig. 4.1.76-4.1.79) The values (Table 4.1.19 and 4.1.21) obtained by regression analysis provide information regarding the complex formation of drug hydrotropes systems.

The negative free energies of solubilization process are indicative of spontaneity of the process, more negative the free energy of complexation, the greater the solubility. The different value of ΔG corresponding to stability constants indicates different solubilization mechanism. The comparison of enthalpy and entropy changes to the free energy change of two type of complex formation also displayed considerable differences. The difference between free energy and enthalpy changes reflects the strength of, interaction or bonding strength of complexion. The value were found to be greater for 1:2 complex than 1;1 complex showing that the former is stronger than latter. The values of bonding strength (enthalpy change) indicate possibility of hydrogen bond in interaction between solubilizer and solutes. However, considering that hydrogen bond are designated as weak (<13 kJ/mol), normal (13-42 kJ /mol) or strong (>42 kJ /mol). The strength of these estimated ΔH value for both the drugs were not strong (Table 4.1.19 and 4.1.21), hence it can be speculated

that intermolecular forces other than hydrogen bonding are involved in solubility enhancement of chlorzoxazone and indomethacin.

The hydrotropic solubilization was found primarily to be entropy driven process at high hydrotrope concentration that was accomplished by very small free energy changes and heat changes and large entropy change. The value shows involvement of weak hydrotropic interactions. This can be explained by flickering cluster hypothesis, which states that water molecules arrange around a polar molecule in flickering clusters.

Stripping the weak molecule from the solute results in randomization of water molecule and a large entropy change. Application of this assumption to hydrotropic solubilization can be described as transfer of solute from aqueous to hydrotrope aggregates or stack at higher concentrations. However the role of water is stabilizing these complexes, hence enthalpy contribution cannot be ignored which is also reflected in enthalpy values for 1:1 and 1:2 complexes. The thermodynamic discussion in this study may have limitation but the interpretation of data can prove useful for extension of knowledge in this field.

On the basis of data obtained, the overall solubility increase can be differentiated in the following two patterns: Solubility at lower hydrotrope concentration and solubility at higher hydrotrope concentration. Some literature subdivided the force controlling interaction between ligand and substrate into 5 categories^{14, 35, 36, 37}:

- 1. Electrostatic forces
- 2. Inductive effects
- 3. Hydrogen bonding
- 4. Charge transfer (electron donor acceptor) interactions
- 5. Hydrophobic effects

Electrostatic forces are generated among ions and molecules possessing permanent dipole moments and induction forces arise as a result of the interaction of an ion or polar molecules with the neighboring molecule. Interaction through hydrogen bonding is important in water.

The increased solubility of investigated drugs were possible due to weak ionic interactions and hydrogen bonding; these interactions are small in magnitude and attributed solubility enhancements at lower concentrations. Jain and group³⁸⁻⁴⁰ have reported these as controlling factor for hydrotropic solubilization at low ligand concentration.

The solubility at higher hydrotrope concentration is result of different kind of interactions both hydrophobic and donor acceptor forces are frequently larger in magnitude and may be involved as controlling factors in higher order water soluble complex formation the result of spectrum study suggest charge transfer interaction is not determinant of complex formation. The investigation revealed involvement of ligand hydrophobicity¹⁶ as an important factor in complexation and stacking hydrotropes through its planarity, and strongly supports the assumption that ligand hydrophobicity is directly related to the degree of hydrotropic solubilization. The studies suggest solubilizer exert their hydrotropic action not via direct interactions with solute, but rather by altering the nature of water as a solvent.

Finally it can be concluded that the solubilization of drugs cannot be attributed to complexation. The hydrotrope self-association significantly plays a role in solubilization mechanism. Additionally, high concentration of hydrotropic in conjugation with self-association change the solvent nature of water and hydrotropic system behaves as a co-solvent system in which one component is solid in solution rather than liquid.

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4.2 COMPLEXATION SOLUBILIZATION

The solubility of non-polar, poorly soluble drugs can be altered in many ways, among them cyclodextrins have emerged as very effective additive compounds for solubilizing hydrophobic drugs. The current status of solubilizers in parenteral products considerably limits the scope of applications for this route of drug delivery. On the other hand, inclusion complexes with cyclodextrin derivatives are well accepted and can even prevent intolerance reactions due to the active substance itself. In recent years several chemically modified β -cyclodextrins, including hydroxypropyl β -cyclodextrins (HP β -CD) and sulphobutyl ether- β -cyclodextrin (SBE β .CD) were found to have greatly increase water solubility and reduced renal toxicity. In the parenteral dosage form area, modified cyclodextrins, such as hydroxypropyl- β -cyclodextrin and sulfobutylether- β -cyclodextrin have been reported to solubilize and stabilize many injectable drugs, including dexamethasone, estradiol, injecterleukin-2 and other proteins and peptides without apparent compatability problems.

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. In aqueous solutions cyclodextrins are able to solubilize lipophilic water insoluble drugs by taking some lipophilic moiety of the drug molecule into the central cavity. Such complexes are called inclusion complexes. However, these cyclic oligosaccharides are also known to form water soluble non-inclusion complexes with lipophilic water insoluble compounds. Cyclodextrins and cyclodextrin complexes are known to self-associate to form aggregates or micelle like structures consisting of two to several hundred cyclodextrin molecules and/or cyclodextrin complexes.

4.2.1 Phase Solubility Study of Chlorzoxazone in hydroxypropyl β -cyclodextrin solution

The phase solubility experiment was performed by the method reported by Higuchi and Connors¹¹. Samples were prepared in triplicate by adding 20 ml of hydroxypropyl β -cyclodextrin solution of successively increasing concentration i.e. 1.0%, 2.0%, 3.0%, 4.0%, 5.0%, 8.0%, 10.0% and 20.0% into a series of 30 ml screw capped culture tubes. Excess quantity of

chlorzoxazone was added into each tube to maintain saturated condition. Each tube was capped and shaked vigorously for 15 minutes on touch type vortex mixer (Jyoti Scientific Industries Gwalior-474 009, India) and then the solutions were allowed to equilibrate with mechanically shaking and intermittent vortexing for 72 hrs at 25±2°C and 37±2°C in a rotary flask shaker and shaker water bath (Jyoti scientific Industries Gwalior-474 009, India). After completion of 72 hrs, each culture tube is centrifuged for 10 min at 2000 rpm. The supernatant of each culture tube was filtered through 0.45 μ membrane syringe filter (Sonar Axiva, Axiva Sichem Pvt. Ltd. Delhi, India.), filtrate diluted suitably with distilled water and analyzed spectrophotometrically at 280 nm against hydroxypropyl β -cyclodextrin solution diluted accordingly as blank. The solubility of chlorzoxazone was determined in triplicate.

Solubility of chlorzoxazone in mg/ml was calculated in different concentration of hydroxypropyl β -cyclodextrin solution and shown in table 4.2.1 and graphically presented in figure 4.2.1. Solubility enhancement ratio was also calculated and reported in the same table and graphically presented in figure 4.2.3.

4.2.2 Phase Solubility Study of Indomethacin in hydroxypropyl β -cyclodextrin solution

Similarly the phase solubility experiment of Indomethacin was performed by the same method as used for chlorzoxazone. An excess quantity of indomethacin was equilibrated with the hydroxypropyl βcyclodextrin solution of various concentrations for 72 hrs at 25±2°C and 37±2°C then centrifuged and filtered through 0.45μ membrane syringe filter. The filtrate diluted suitably with distilled water and analyzed 319.5 at nm against hydroxypropyl spectrophotometrically β-cyclodextrin solution diluted accordingly as blank. The solubility of Indomethacin was determined in triplicate.

Solubility of Indomethacin in mg/ml was calculated in different concentration of hydroxypropyl β -cyclodextrin solution and shown in table 4.2.2 and graphically presented in figure 4.2.2. Solubility enhancement ratio was calculated and reported in the same table and graphically presented in figure 4.2.4.

Table 4.2.1: Solubility data of chlorzoxazone in various concentration of HP- β -CD solution

S. No.	%HP-β-CD	Solubility	in mg/ml	Enhancement Ratio		
		At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C	
1.	0.00%	0.318 ± 0.013	0.347 ± 0.012	1.000	1.000	
2.	1.00%	2.550 ± 0.233	2.780 ± 0.215	8.009	8.006	
3.	2.00%	3.272 ± 0.307	3.503 ± 0.285	10.279	10.086	
4.	3.00%	3.964 ± 0.539	4.286 ± 0.527	12.452	12.343	
5.	4.00%	4.778 ± 0.389	4.917 ± 0.451	15.011	14.158	
6.	5.00%	5.654 ± 0.473	6.131 ± 0.428	17.763	17.654	
7.	8.00%	7.545 ± 0.747	7.729 ± 0.643	23.702	22.257	
8.	10.00%	8.790 ± 0.817	9.134 ± 0.784	27.613	26.302	
9.	20.00%	14.584 ± 0.991	15.184 ± 1.029	45.816	43.724	

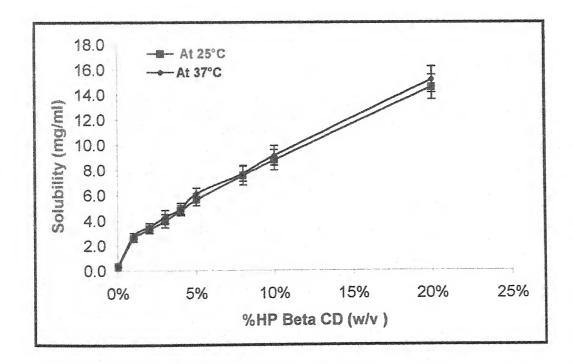


Fig. 4.2.1: Solubility plot of chlorzoxazone in HP- β -CD

Table 4.2.2: Solubility data of indomethacin in various concentration of HP- β -CD solution

S. No.	%HP- β -CD -	Solubility	in mg/ml	Enhancement Ratio		
		At 25±2°C	At 37±2°C	At 25±2°C	At 37±2°C	
1.	0.00%	0.025 ± 0.005	0.028 ± 0.007	1.000	1.000	
2.	1.00%	0.114 ± 0.047	0.126 ± 0.037	4.548	4.433	
3.	2.00%	0.186 ± 0.037	0.202 ± 0.045	7.464	7.113	
4.	3.00%	0.273 ± 0.052	0.293 ± 0.060	10.938	10.312	
5.	4.00%	0.310 ± 0.047	0.327 ± 0.052	12.413	11.523	
6.	5.00%	0.339 ± 0.062	0.339 ± 0.072	13.593	11.955	
7.	8.00%	0.484 ± 0.087	0.524 ± 0.079	19.384	18.440	
8.	10.00%	0.796 ± 0.052	0.850 ± 0.060	31.878	29.940	
9.	20.00%	2.095 ± 0.106	2.323 ± 0.118	83.884	81.819	

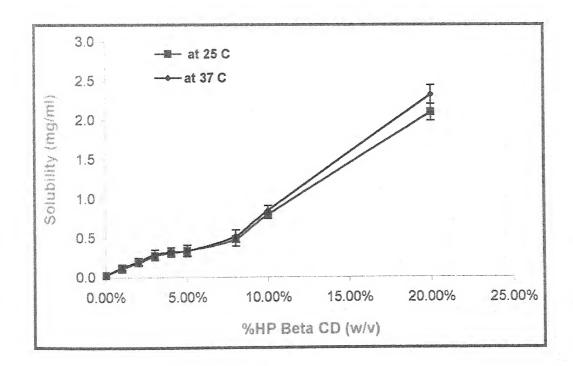


Fig. 4.2.2: Solubility plot of indomethacin in HP- β -CD

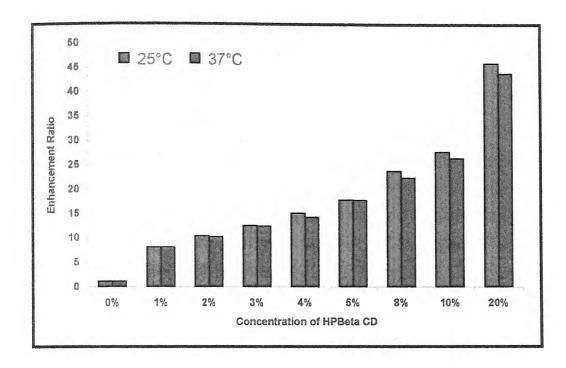


Fig. 4.2.3: Solubility enhancement plot of chlorzoxazone in HP-β-CD

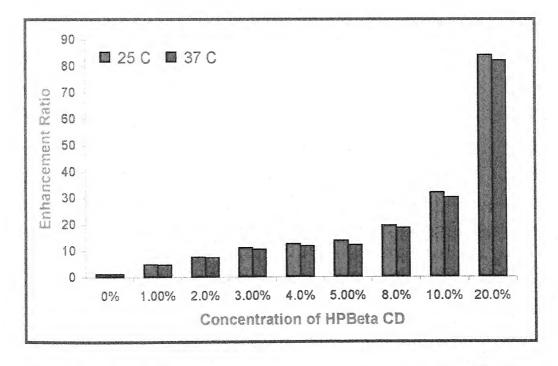


Fig. 4.2.4: Solubility enhancement plot of indomethacin in HP- β -CD

4.2.3 Mechanistic Studies

In order to understand and interpret probable mechanism of solubilization of drug with hydroxypropyl β-cyclodextrin UV spectral studies. FTIR studies, thermal analysis and XRPD were performed.

4.2.3.1 UV Spectral Studies

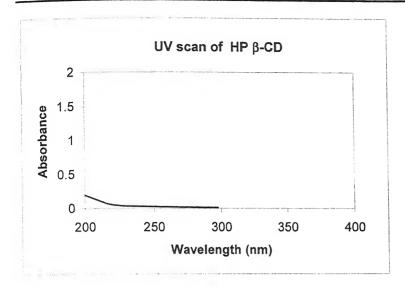
1 ml of stock solution of the drug (Section 3.2.1.1 and 3.2.2.1) was diluted to 10 ml with 10 mg/ml solution hydroxypropyl β-cyclodextrin. The resultant solution was scanned in UV range from 200 to 400 nm using distilled water as blank by Simadzu-1701 (Japan) UV spectrophotometer. Any shift in λ_{max} of the drug was noted and shown in table 4.1.1. The spectrum were also recorded and presented in figure 4.2.5.

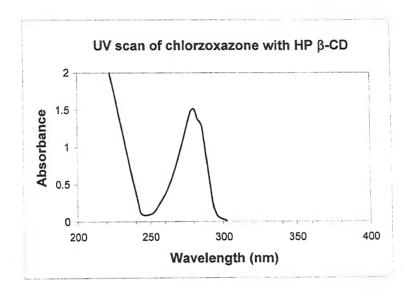
4.2.3.2 FTIR Spectral Studies

FTIR spectral studies of physical mixture of drug (chlorzoxazone or indomethacin) with hydroxypropyl β-cyclodextrin and their complexes were performed using FTIR-spectrophotometer (FTIR-8400s Shimadzu, Japan). All samples were dried in vacuum for 24 hrs before FTIR studies. 5 mg of each sample was mixed with about 100 mg of potassium bromide (vacuum dried for 24 hrs) and compressed as pallet. Measurements were attempted with accumulation of 20 scans and a resolution of 4 cm⁻¹ over a range of 400 to 4000 cm⁻¹ (Fig. 4.2.6-4.2.10).

4.2.3.3 Thermal Analysis

Thermal analysis of physical mixture of the drug (chlorzoxazone or indomethacin) and hydroxypropyl β -cyclodextrin and their complex were performed by differential scanning calorimetry, using pyres-6 DSC (Perkin Elmer, USA). Samples were prepared by placing 5 mg. of the drug substance in to an aluminum pan, which covered and crimped for analysis. Samples were desiccated over calcium chloride for 24 hours prior to assay in an effort to remove surface absorbed water. Thermograph was analyzed qualitatively by examining both the peak temperature and the endothermic transition contour. The nitrogen flow rate was 20 ml/min and the heating rate was 5°C/min over the range of 40 to 300°C. The thermographs are shown in figure 4.2.11-4.2.13.





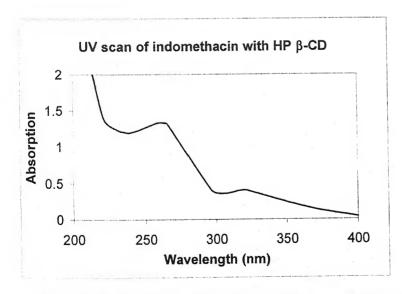


Fig. 4.2.5: UV spectral study of chlorzoxazone and indomethacin interaction with HP- β -CD

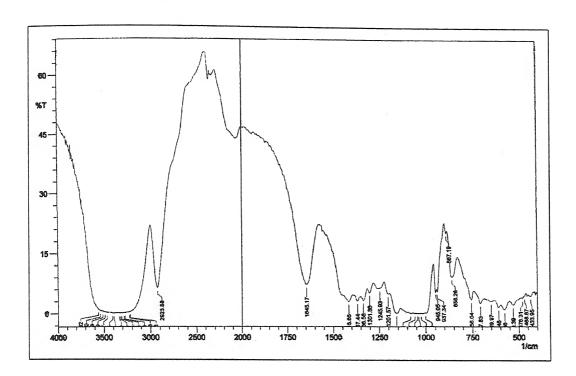


Fig. 4.2.6: FTIR spectrum of hydroxypropyl-β-cyclodextrin

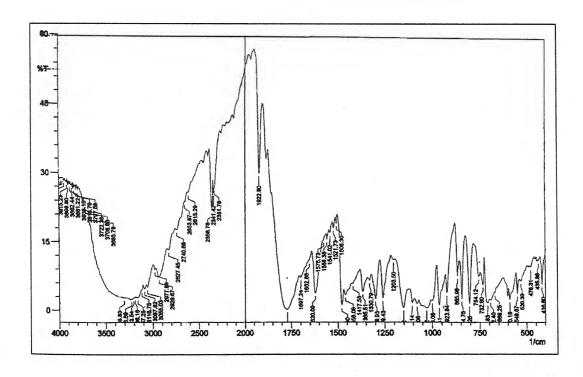


Fig. 4.2.7: FTIR spectrum of chlorzoxazone+hydroxypropyl-β-cyclodextrin (PM)

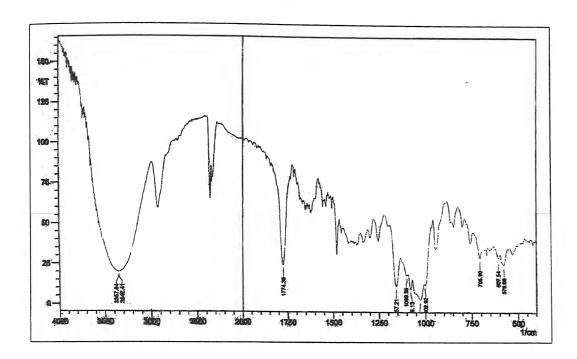


Fig. 4.2.8: FTIR spectrum of chlorzoxazone+hydroxypropyl-β-cyclodextrin (Solubilized)

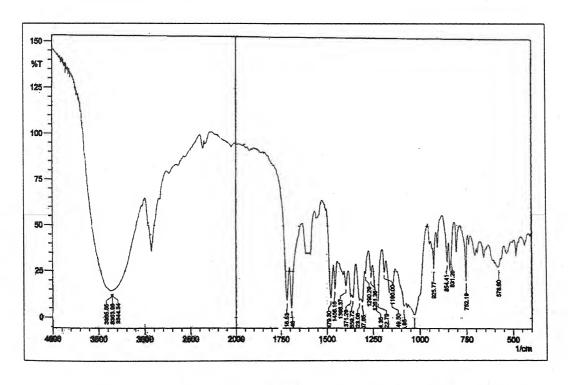


Fig. 4.2.9: FTIR spectrum of indomethacin+hydroxypropyl- β -cyclodextrin (PM)



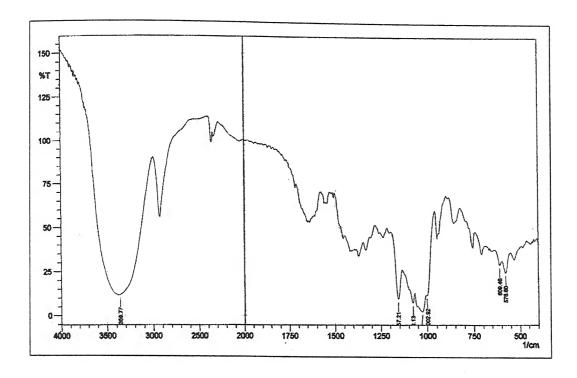


Fig. 4.2.10: FTIR spectrum of indomethacin+hydroxypropyl-βcyclodextrin (Solubilized)

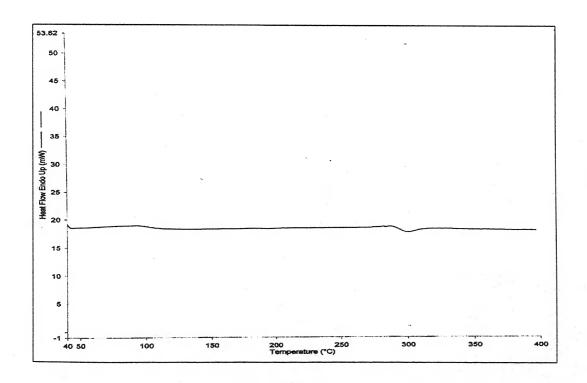


Fig. 4.2.11: DSC curve of hydroxypropyl-β-cyclodextrin

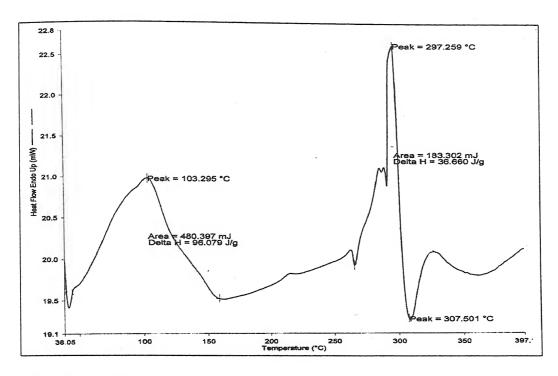


Fig. 4.2.12: DSC curve of chlorzoxazone+hydroxypropyl-β-cyclodextrin (Solubilized)

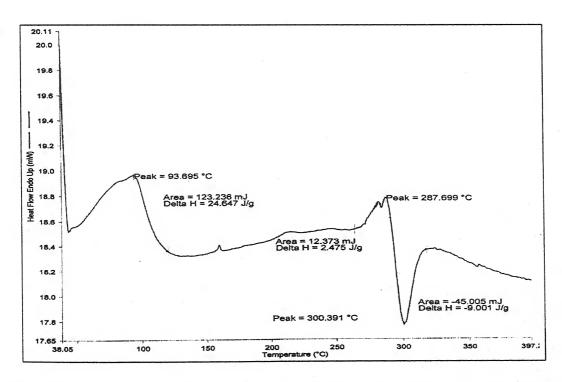


Fig. 4.2.13: DSC curve of indomethacin+hydroxypropyl- β -cyclodextrin (Solubilized)

4.2.3.4 X-Ray Powder Diffraction

X-ray diffraction patterns of physical mixture of the drug (chlorzoxazone or indomethacin) and hydroxypropyl β-cyclodextrin and their complex were obtained at room temperature (25°C) using a D-8 Advance, Bruker-AXS Diffractometer (Germany). Samples were exposed to Cu K α radiation at scanning rate 2°/min with step size 0.050°, step time 1.5 second over scanning range 3.000° to 120.000° of the diffraction angle 26; the generator was set to 40 kV and 30mA. X-ray diffraction patterns are shown in figure 4.2.14-4.2.18.

4.2.3.5 Microscopic studies

The Hydroxypropyl β-cyclodextrin and their complexes with drugs were microscopically for their crystallinity and morphological examined characteristics and recorded by photomicrograph using Ezee capture camera. The photomicrographs are shown in plate-4.

4.2.4 Mathematical Analysis of Phase Solubility Data

The stoichiometry of drug/cyclodextrin complexes and the numerical values of their stability constants are frequently obtained from phase-solubility diagrams, i.e., plots of drug solubility versus cyclodextrin concentration. Linear phase-solubility diagrams (A_L -type systems) indicate that the complex is first order with respect to cyclodextrin and first or higher order with respect to the drug (D):

$$mD + CD \leftrightarrow D_mCD$$
 ...(1)

In this case, the total drug solubility (Stot) will be given by:

$$S_{tot} = S_W + m[D_m \cdot CD] \qquad ...(2)$$

Where S_W is the inherent solubility of the drug in the aqueous complexation medium. If one drug molecule forms a water-soluble complex with one cyclodextrin molecule (i.e., 1:1 inclusion complex), then the slope of the linear phase-solubility diagram will be determined by the equation:

$$Slope = \frac{S_W K_{1:1}}{S_W K_{1:1} + 1} ...(3)$$

Where $K_{1:1}$ is the stability constant for the complex. In this case, the slope is always less than unity. If 2:1 drug/cyclodextrin complex is formed,

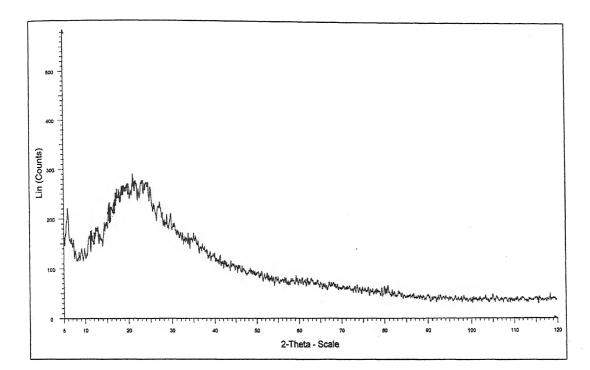


Fig. 4.2.14: XRPD of hydroxypropyl-β-cyclodextrin

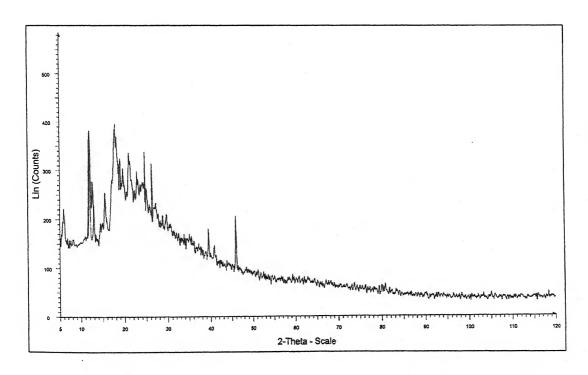


Fig. 4.2.15: XRPD of chlorzoxazone+hydroxypropyl- β -cyclodextrin (PM)

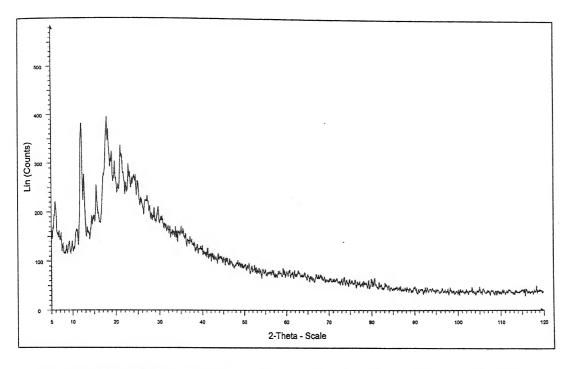


Fig. 4.1.16: XRPD of chlorzoxazone+hydroxypropyl- β -cyclodextrin (Solubilized)

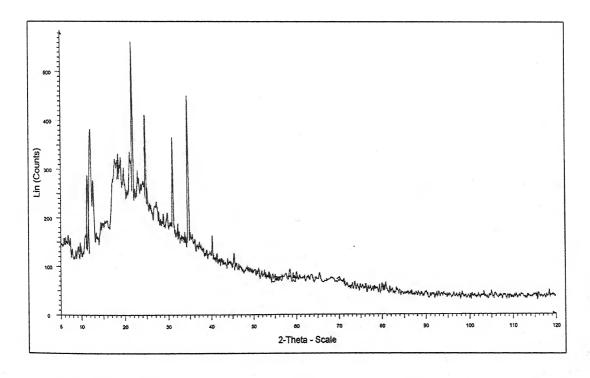


Fig. 4.2.17: XRPD of indomethacin+hydroxypropyl-β-cyclodextrin (PM)

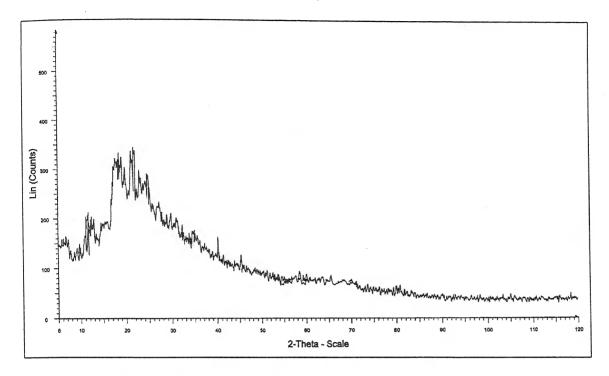


Fig. 4.2.18: XRPD of indomethacin+hydroxypropyl-β-cyclodextrin (Solubilized)

then the slope of the linear phase solubility diagram will be determined by the equation:

$$Slope = \frac{2S_W^2 K_{2:1}}{S_W^2 K_{2:1} + 1} \qquad ...(4)$$

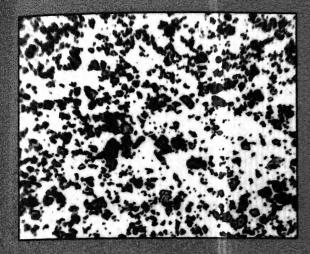
Where $K_{2:1}$ is the stability constant of the complex. In this case, the slope of the linear phase-solubility diagram is always less than two. Positive deviation from linearity (Ap-type phase-solubility diagrams) suggests formation of a higher-order complex with respect to cyclodextrin. The stoichiometry of the system can be probed by curve fitting with a quadratic model suggesting formation of a 1:2 drug/cyclodextrin complex:

$$S_{tot} = S_W + K1:1S_W[CD] + K_{1:1}K_{1:2}S_W[CD]^2$$
 ...(5)

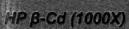
Where [CD] represents the concentration of free cyclodextrin. A third-order model is suggestive of a 1:3 complex, etc.

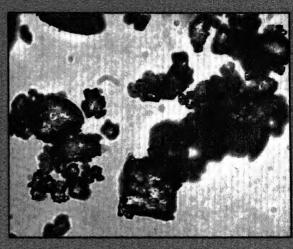
The total solubility of a drug (S_{tot}) in aqueous surfactant solutions is generally described by the following equation (6)

$$S_{tot} = S_W + k(C_{surf} - CMC) \qquad ...(6)$$



HP β-Cd (100X)





Chlorzoxazone-HP β-Cd solubilized (100X)

Indomethacin-HP β-Cd solubilized (400X)

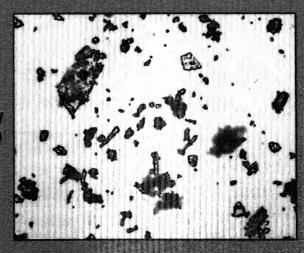


Plate 4: Photomicrograph of HP β-Cd and Drug-HP β-Cd Complex

$$k = \frac{S_M}{C_{\text{mic}}} \qquad ..(7)$$

Where C_{mic} is the molar concentration of micellar surfactant and S_{M} is the drug concentration within the micelles. If the CMC value is much lower than the surfactant concentration, eq. 6 can be simplified to:

$$S_{tot} = S_W + kC_{surf} \qquad ...(8)$$

When drug/cyclodextrin complexes and micelles or aggregates coexist, S_M will be given by:

$$S_M = S_{tot} - S_W - [D . CD]$$
 ...(9)

Where S_{tot} is as before the total concentration of dissolved drug, S_W is as before the inherent solubility of the drug in the pure aqueous medium, and [D.CD] is the concentration of drug/cyclodextrin complex. Here, we assume that only 1:1 drug/cyclodextrin complexes are formed and that the two events, inclusion complex formation and solubilization via non-inclusion complex formation, are independent.

Because the slope in a phase solubility is:

$$Slope = \frac{S_{tot} - S_W}{[CD]_{tot}} \qquad ...(10)$$

and K_{1:1} is:

$$Slope = \frac{1}{S_W} \frac{[CD.D]}{[CD]_{tot} - [CD.D]} ...(11)$$

It can be shown that the slope of a phase-solubility diagram in a system where solubility occurs through both inclusion and association is:

$$Slope = \frac{S_W K_{1:1}}{S_W K_{1:1} + 1}$$
 $(1 + k) = Slope' (1 + k)$...(12)

Slope' is the contribution form inclusion complex formation, which cannot be more than unity but added contribution from association can result in a slope that is greater than unity. These theoretical calculations show that

4.2.5 Estimation of Thermodynamic Parameters for the Stability Constant

The free energy change ΔG is determined from the stability constant K by the expression:

$$\Delta G = -2.303RT \log K$$
 ...(13)

Where R is the gas constant (8.3143 J.mol⁻¹.K⁻¹) and T is the absolute temperature. The enthalpy change ΔH is estimated from the stability constant at several temperatures by the following relationship.

$$\log K = -\frac{\Delta H}{2.303R} \times \frac{1}{T} + \text{const.}$$
 ...(14)

A linear plot of log K against 1/T (a van't Hoff plot) yields ΔH from slope. The entropy change ΔS is related to ΔG and ΔH as:

$$\Delta S = \frac{\Delta H - \Delta G}{T} \qquad ...(15)$$

Table 4.2.3: Solubilization parameters of drug-HP-β-CD interaction

Drug	Temp.	S _W (M)	Slope	Complexation Efficiency	Stability Constant (K _{1:1})
	25	1.877	0.551	1.226	652.901
Chlorzoxazone	37	2.048	0.568	1.315	642.260
	25	0.070	0.041	0.043	615.660
Indomethacin	37	0.079	0.046	0.048	603.464

Table 4.2.4: Thermodynamic parameter for drug-HP- β -CD interaction

Drug	Temp. (°C)	Log K _M	ΔG (kJ.mol ⁻¹)	ΔH (kJ.mol ⁻¹)	∆S (J.mol ⁻¹ .K ⁻¹)
	25	652.91	-16.0582	-1051.22	-3473.68
Chlorzoxazone	37	642.26	-16.0175	-1051.22	-3339.35
	25	615.6603	-15.9127	-1280.99	-4245.23
Indomethacin	37	603.4638	-15.8631	-1280.99	-4081.05

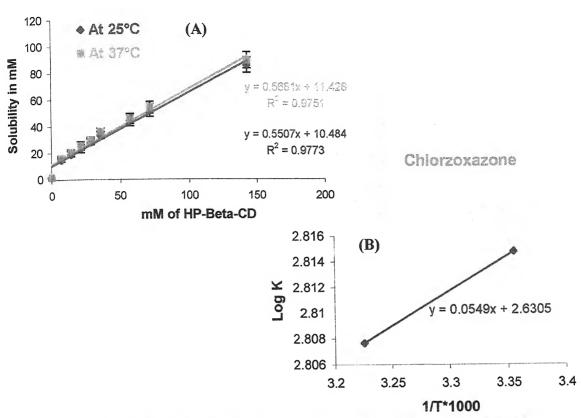


Fig. 4.2.19: (A) Plot for determination of K_{1:1} (B) von't Hoff plot for chlorzoxazone in HP-β-CD interaction

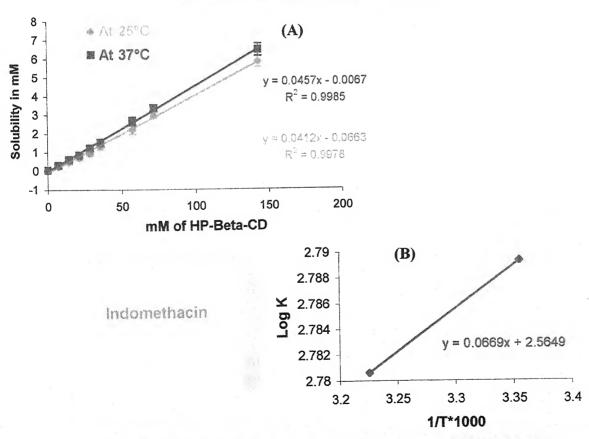


Fig. 4.2.20: (A) Plot for determination of $K_{1:1}$ (B) von't Hoff plot for indomethacin in HP- β -CD interaction

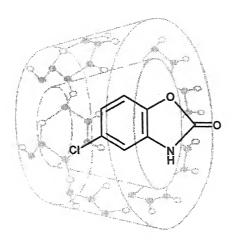


Fig. 4.2.21: Possible interaction of chlorzoxazone with HP- β -CD in aqueous solution

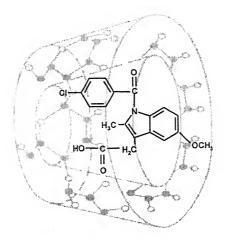


Fig. 4.2.22: Possible interaction of indomethacin with HP- β -CD in aqueous solution

The effect of hydroxypropyl β -cyclodextrin (HP β -CD) on aqueous solubility of chlorzoxazone and indomethacin was evaluated using the phase solubility method ¹¹. Figure 4.2.19(A) and 4.2.20(A) showed the phase solubility diagram of chlorzoxazone and indomethacin respectively with various concentrations of hydroxypropyl β -cyclodextrin. The phase diagram was classified as type A_L by Higuchi ¹¹, which denotes a linear increase in solubility.

Increase in chlorzoxazone solubility in aqueous hydroxypropyl β-cyclodextrin solution was consistent with the formation of inclusion complexes between chlorzoxazone and hydroxypropyl β-cyclodextrin molecule. In general the main driving force for the complex formation is the hydrophobic interaction between poorly soluble quest compound, apolar cavity of hydroxypropyl chlorzoxazone and the β-cyclodextrin molecule. The hydrophobicity and geometry of the guest molecule are important parameters for the complex formation. The solubility enhancement attained by 20% W/V hydroxypropyl β-cyclodextrin was 14.584 and 15.184 mg/ml at 25°C and 37°C respectively that was 45.8 fold and 43.724 fold, of aqueous solubility of Chlorzoxazone at the respective temperature. Based on phase solubility diagram, the association constants for the inclusion complex was determined assuming 1:1 ratio of complex formation¹³.

Slopes and intercepts were determined by performing linear regression analysis of phase solubility data (Fig. 4.2.19A). From figure the regression equations for calculation of $K_{1:1}$ at various temperature were found to be Y $(25^{\circ}) = 0.5507x + 10.484$ and Y $(37^{\circ}) = 0.5681x + 11.428$. Association constants $K_{1:1}$ at both temperature were computed using the equation (3). The value of $K_{1:1}$ were found to be 652.91 M^{-1} and 642.26 M^{-1} at 25 °C and 37°C respectively. For 1:1 chlorzoxazone/ hydroxypropyl β -cyclodextrin complex, the complexation efficiency is defined by the following equation:

Complexation efficiency =
$$S_W.K_{1:1} = \frac{[D/CD]}{[CD]} = \frac{Slope}{1 - Slope}$$
(16)

i.e., the concentration of the drug/cyclodextrin complex ([D/CD]) divided by concentration of free cyclodextrin ([CD])¹⁴. The slope in equation 16 represents the slope of the phase-solubility diagram. The complexation efficiency of hydroxypropyl β -cyclodextrin was found to be 1.226 and 1.315 at 25°C and 37°C respectively (Table 4.2.3).

The complex stability decreases with increase of temperature. Similar observations have been reported concerning the stability of inclusion complexes of hydroxypropyl β-cyclodextrin with several other drug molecule 15,16. The value indicates the solubilization capacity of hydroxypropyl β-cyclodextrin decreased as temperature increased. Cyclodextrin and cyclodextrin complexes are know to self-associate to form aggregates or micelle structures consisting of two or several hundred cyclodextrin molecules and/or cyclodextrin complexes 10 and these aggregates can solubilize lipophilic water insoluble drugs through non-inclusion complexation 9. These aggregates dissociate upon heating, increasing the relative concentration of complex monomers, thus reducing the aqueous solubility of water insoluble drugs 17.

van't Hoff plot was constructed to estimate thermodynamic parameters of chlorzoxazone-hydroxypropyl β -cyclodextrin complex (Fig. 4.2.19B). The Thermodynamic parameters were shown in table 4.2.4. The values of entropy change ΔS (disordering or bond breaking) were negative, revealing the possibility of an increased ordering of species by complexation. Both values of the free energy change ΔG and the enthalpy change ΔH (bonding strength) were negative, indicating the spontaneity and exothermic nature of chlorzoxazone-hydroxypropyl β -cyclodextrin complexation solubilization. Large negative ΔH suggests that chlorzoxazone hydroxypropyl β -cyclodextrin inclusion is an enthalpy driven process¹⁸. The negative value obtained for both ΔH and ΔS would seem to suggest that stabilizing interactions in the complex were hydrophobic¹⁹ as well as through hydrogen bonding.

Similar to chlorzoxazone, increasing the amounts of hydroxypropyl β -cyclodextrin increased the amount of indomethacin going into water, improving aqueous solubility of indomethacin. The phase solubility diagram was found to be A_L type¹¹ shown in figure 4.2.3 suggesting 1:1 stoichiometry of the inclusion complex¹³.

The solubility enhancement attained by 20% W/V hydroxypropyl β -cyclodextrin was 2.095 and 2.323 mg/ml at 25°C and 37°C respectively that was 83.884 fold and 81.819 fold of aqueous solubility of indomethacin at the respective temperature. Based a phase solubility diagram, the association constants for the inclusion complex was determined assuming 1:1 ratio of complex formation.

Slopes and intercepts were determined by performing linear regression analysis of phase solubility data (Fig. 4.2.20A). From figure the regression equations for calculation of $K_{1:1}$ at various temperature were found to be Y $(25^{\circ}) = 0.0412x - 0.0663$ and Y $(37^{\circ}) = 0.0457x - 0.0067$. Association constants $K_{1:1}$ at both temperature were computed using the equation (3). The value of $K_{1:1}$ were found to be 615.6603 M^{-1} and 603.4638 M^{-1} at 25°C and 37°C respectively. For 1:1 indomethacin-hydroxypropyl β -cyclodextrin complex, the complexation efficiency of hydroxypropyl β -cyclodextrin was found to be 0.043 and 0.048 at 25°C and 37°C respectively (Table 4.2.3).

Similar to chlorzoxazone the complex stability decreases with increase of temperature.

van't Hoff plot was constructed to estimate thermodynamic parameters of indomethacin-hydroxypropyl β -cyclodextrin complex (Fig. 4.2.20B). The Thermodynamic parameters were shown in table 4.2.4. The values of entropy change ΔS (disordering or bond breaking) were negative, revealing the possibility of an increased ordering of species by complexation. Both values of the free energy change ΔG and the enthalpy change ΔH (bonding strength) were negative, indicating the spontaneity and exothermic nature of indomethacin- hydroxypropyl β -cyclodextrin complexation solubilization. Large

negative ΔH suggests that indomethacin-hydroxypropyl β-cyclodextrin inclusion is an enthalpy driven process¹⁸. The negative value obtained for both ΔH and ΔS would seem to suggest that stabilizing interactions in the complex were hydrophobic 19 as well as through hydrogen bonding.

Reduced to its elementary stages, inclusion complexing occurs as a sequence of entropically opposing partial steps²⁰. Displacement of water molecules from the cavity, stripping of the shell of hydration from the incoming molecule to be complexed, and hydration of cyclodextrin itself are processes. which directly affect the degree of ordering of the water. The inclusion complex formation process itself must therefore depend on the state of ordering of the water and react to enforced changes in the entropy of the system. The sum of the entropy changes enters via the entropy term into the change in free energy and determines the course of establishment of the complex equilibrium from a thermodynamic point of view.

In order to interpret the mechanism of complexation UV spectrum, FTIR studies, DSC, XPRD studies were performed.

The UV spectra of chlorzoxazone in hydroxypropyl β-cyclodextrin solution showed no change in λ_{max} (280.0). The hydroxypropyl β -cyclodextrin it self showed insignificant ultra violet absorbance (Fig. 4.2.5).

The infrared spectrum of chlorzoxazone hydroxypropyl β-cyclodextrin complex was similar to the pure hydroxypropyl β -cyclodextrin but dissimilar to chlorzoxazone and a physical mixture of chlorzoxazone and hydroxypropyl β -cyclodextrin as shown in the figure 4.2.6-4.2.8. Miner peaks and peak shifts were observed in the complex spectrum when comparison made to the pure hydroxypropyl β-cyclodextrin.

The hydroxypropyl β-cyclodextrin -chlorzoxazone complex showed a peak at 2358 cm⁻¹, which was due to resonance hydroxyl-carbonyl group of chlorzoxazone suggesting that this group was not covered by the hydroxypropyl β-cyclodextrin ring cavity.

The complex also showed a broad peak at about 1645.17 cm⁻¹ when compared to sharp peak of pure hydroxypropyl β-cyclodextrin at 1645.17 cm⁻¹. This broadening of peak was attributed to the change in the hydrated bond within hydroxypropyl β-cyclodextrin cavity. The complex had another peak at 1774.39 cm⁻¹, which was attributed to original 1770.53 cm⁻¹ peak of carbonyl stretch of pure chlorzoxazone. This suggested that carbonyl group of chlorzoxazone was not included within cyclodextrin ring cavity. The possible interactions are shown in figure 4.2.21.

A comparison among the endothermal transitions of chlorzoxazone, the pure HP β -cyclodextrin and chlorzoxazone-hydroxypropyl β -cyclodextrin complex showed the complex thermograms have shifted-peak temperatures as evidence of complex formation as shown in figure 4.2.11-4.2.12 The peak shift occurred in the large, broad endotherm at about (50-150°C) that was attributed to dehydration of hydroxypropyl β -cyclodextrin ring cavity. The hydroxypropyl β -cyclodextrin complex was dissimilar to physical mixture, thus suggesting complex formation. The complex showed no evidence of sharp chlorzoxazone response at 194.399°C.

X-ray diffraction patterns of chlorzoxazone showed crystalline reflections indicating chlorzoxazone was crystalline powder. While X-ray diffraction patterns of hydroxypropyl-β-cyclodextrin and its complex with chlorzoxazone showed that they were amorphous as depicted in figure 4.2.14-4.2.16. The hydroxypropyl-β-cyclodextrin complex was dissimilar to physical mixture, thus suggesting complex formation²¹. Complex formation could not be confirmed because their amorphous nature precluded differantiation by crystalline reflections.

The UV spectra of indomethacin in HP β -Cyclodextrin solution (Fig. 4.2.5) showed that there was no shift of the two λ_{max} (319.5 and 265) of indomethacin when complexed with the hydroxypropyl β -cyclodextrin however a increase in the absorbance of indomethacin was observed. The hydroxypropyl β -cyclodextrin itself showed insignificant ultraviolet absorbance at these λ_{max} .

The infrared spectrum of the hydroxypropyl β -cyclodextrin complex was similar to the pure hydroxypropyl β -cyclodextrin but aissimilar to indomethacin and physical mixture of indomethacin and hydroxypropyl β -cyclodextrin as shown in figure 4.2.9-4.2.10 minor peaks and peak shift were observed in the complex spectra when comparison made to the pure hydroxypropyl β -cyclodextrin.

The hydroxy propyl β -cylodextin indomethacin complex showed a broad peak between 1640-1670 compared to sharps peak at 1645.17 cm⁻¹ for hydroxypropyl β -cyclodextrin. This broadening of 1645.17 cm⁻¹ peak was attributed to the change due to hydrated bonds within hydroxypropyl β -cyclodextrin. The complex also had a broad peak at to about 1554 to 1580 cm⁻¹ which was attributed to C=C that stretch absorption of indomethacin, suggested that aromatic ring of indomethacin was not fully covered within the hydroxypropyl β -cyclodextrin cavity.

The complex also an increase in the relative peak intensity of the 1330 cm⁻¹ that was attributed to a C-H bending absorption of indomethacin, which suggested this functional group was not fully included within the cyclodextrin cavity.

In the complex spectrum the lack of the two intense carbonyl bands of indomethacin at 1691.46 cm⁻¹ and 1712.67 cm⁻¹ suggested the inclusion of the two carbonyl groups in the cyclodextrin ring cavity and bound well to the cyclodextrin cavity.

Considering the infrared spectrum, change in the water absorbance at 1645.17 cm⁻¹ was found as evidence of complex formation. The C–H bonding and C=C stretching absorbencies suggested the methoxy group and the adjacent aromatic ring of indomethacin were not included fully within the cyclodextrin ring cavity. The possible interactions are shows in figure 4.2.22.

A study of thermal transitions of indomethacin, hydroxypropyl β -cyclodextrin, physical mixture and their complex revealed complex formation as evident from shift in peak temperature in the complex thermo gram

(Fig. 4.2.13). The peak shift occurred in the large, broad endotherm at about 93°C that was attributed to the dehydration of the hydroxypropyl β -cyclodextrin ring cavity. The hydroxypropyl β -cyclodextrin complex was dissimilar to a physical mixture and had no evidence of a strong indomethacin response at 161°C from γ -from of indomethacin, thus suggesting complex formation²¹. It was postulated that the small transitions attributed to indomethacin were caused by small quantities of uncomplexed indomethacin.

Analysis of X-ray diffraction patterns of indomethacin, hydroxypropyl β -cyclodextrin, physical mixture and their complex (Fig. 4.2.17-4.2.18) showed indomethacin was a γ -form crystalline powder evident from characteristic crystalline reflection. hydroxypropyl β -cyclodextrin and its indomethacin complex were amorphous. The hydroxypropyl β -cyclodextrin complex was dissimilar to physical mixture suggesting complex formation, but it could not be confirmed because its amorphous nature precluded differentiation by crystalline reflections.

Finally it is concluded that the solubility enhancement of chlorzoxazone or indomethacin was attributed to inclusion type complex formation of 1:1 stoichiometry evident by the A_L type of Phase Solubility Diagram. The stoichiometry of chlorzoxazone / hydroxypropyl β -cyclodextrin or indomethacin / hydroxypropyl β -cyclodextrin complexes cannot be derived exclusively from simple phase solubility studies self-association of surface active drugs, lipophylic drug molecules and drug / cyclodextrin complexes as well as solubilization through non-inclusion interactions with drug / cyclodextrin complexes, will influence both the shape and mathematical interpretation of the phase solubility diagrams. The results support previous observations made by other investigators that drug / cyclodextrin complexes can self-associate to form water-soluble aggregates of several drug / cyclodextrin complexes. These aggregates can further solubilize the drug through non-inclusion complexation.

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4.3 COSOLVENT SOLUBILITY

If an insoluble drug is to be administered parentally or as an oral liquid it is necessary to find a mean of increasing its aqueous solubility. The most fruitful means of increasing aqueous solubility is to develop an aqueous vehicle, which more closely matches the polarity of drug than pure water¹. This alteration of polarity of aqueous system can effectively achieve by addition of cosolvents. Cosolvents are water miscible organic solvents, which can increase the solubility of a non-polar drug upto several orders of magnitude above the aqueous solubility and enhance the chemical stability of drug²⁻⁴. Thus cosolvent addition is highly effective technique for enhancement of solubility of poorly soluble drugs⁵⁻⁷. The small non-polar hydrocarbon region in the sosolvent can reduce the ability of the aqueous system to squeeze out non-polar solutes⁸.

In the present section, we examined the solubility enhancement of the both drugs in five cosolvents namely propylene glycol, ethanol, glycerin, PEG-200 and PEG-400 and solvent mixtures namely PG-ethanol, PEG-200-ethanol, PEG-400-ethanol and PEG200-PEG400. The selection of these cosolvents were based on availability, inexpensiveness, stability, non-toxins, compatibility and usability in parenteral formulation and well tolerability to patient. The study was conducted to determine how well the particular drugs can be solubilize by these cosolvents and how much cosolvent would be required to attain a specified solubility.

Additionally, the solubility of both drugs was also determined in benzyl alcohol. Though benzyl alcohol is not normally regarded as a cosolvent, this excipient is used in numerous parenteral products as a preservative^{9,10}. Benzyl alcohol is only partially soluble. Its aqueous solubility is approximately 40 mg/ml¹¹, because of its remarkable solubilizing power, the solubilization approach focused on the use of benzyl alcohol as the primary solubilizer.

To determine the equilibrium solubility of chlorzoxazone, the phase solubility experiment was performed by the method reported by Higuchi and Connors¹². Cosolvent-aqueous systems were prepared by mixing 0.0, 0.1, 0.2, 0.3,......1.0 volume fraction of each cosolvent in distilled water. Also cosolvent-cosolvent systems namely ethanol/PG, ethanol/PEG-200, ethanol/PEG-400 and PEG-200/PEG-400 were prepared by mixing both respective cosolvent in the proportion of 10:0, 9:1, 8:2, 7:3, 6:4......1:9 and 0:10.

An excess quantity of chlorzoxazone was added to screw capped 15 ml glass culture tubes containing 10 ml of solvent system. The culture tubes were shaken vigorously for 15 minutes on touch type vortex mixer (Jyoti Scientific Industries Gwalior-474 009, India) and then the solutions were allowed to equilibrate with mechanically shaking and intermittent vortexing for 72 hrs at 25±2 °C and 37±2 °C in a Rotary flask shaker and shaker water bath (Jyoti scientific Industries Gwalior-474 009, India). After completion of 72 hrs, each culture tube is centrifuged for 10 min at 2000 rpm. The supernatant of each culture tube was filtered through 0.45 μ membrane syringe filter (Sonar Axiva, Axiva Sichem Pvt. Ltd. Delhi, India.), filtrate diluted suitably with distilled water and analyzed spectrophotometrically at 280 nm against respective solvent system diluted accordingly as blank. The solubility of chlorzoxazone was determined in triplicate.

Additionally the solubility was also determined in various concentration upto 5% of benzyl alcohol by the same method.

Solubility of chlorzoxazone in mg/ml was calculated in different cosolvent system and shown in table 4.3.1-4.3.10 and graphically presented in figure 4.3.1-4.3.6. Comparative semi-log solubility plot in different cosolvent at 25°C is presented in figure 4.3.7.

Solubility enhancement ratios are reported in the same tables and graphically presented in figure 4.3.8. Plots of solubility of chlorzoxazone in cosolvent mix are presented in figures 4.3.9-4.3.12.

Table 4.3.1: Solubility of chlorzoxazone in various concentration of polyethylene glycol-200

S.	Conc. of	Solubility	in mg/ml	Enhancem	ent Factor
No.	PEG-200	At 25°C	At 37°C	At 25°C	At 37°C
1	0%	0.318±0.013	0.347±0.012	1.000	1.000
2	10%	0.583±0.035	0.659±0.031	1.833	1.898
3	20%	0.874±0.037	1.000±0.044	2.747	2.881
4	30%	1.415±0.051	1.592±0.047	4.446	4.585
5	40%	2.644±0.080	2.975±0.132	8.307	8.568
6	50%	5.131±0.239	5.496±0.294	16.118	15.825
7	60%	11.295±0.459	12.294±0.644	35.483	35.402
8	70%	24.728±1.776	27.228±2.944	77.683	78.407
9	80%	57.244±4.166	62.547±4.781	179.830	180.111
10	90%	85.541±6.591	94.672±7.052	268.727	272.618
11	100%	136.756±14.411	150.896±14.718	429.618	434.522

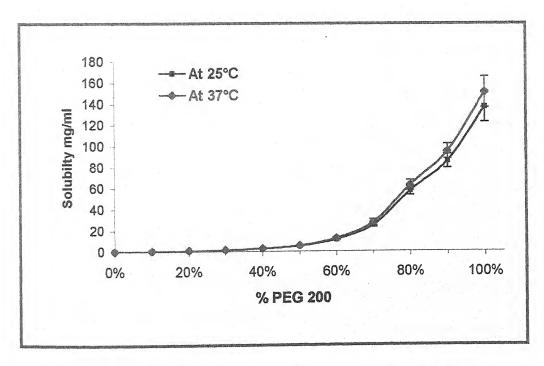


Fig. 4.3.1: Solubility plot of chlorzoxazone in PEG-200

Table 4.3.2: Solubility of chlorzoxazone in various concentration of polyethylene glycol-400

S.	Conc. of	Solubility	in mg/ml	Enhancement Factor	
No.	PEG-400	At 25°C	At 37°C	At 25°C	At 37°C
4	0%	0.318±0.013	0.347±0.012	1.000	1.000
2	10%	0.566±0.044	0.624±0.060	1.778	1.797
3	20%	0.913±0.055	1.010±0.066	2.868	2.907
4	30%	2.034±0.043	2.281±0.147	6.389	6.567
5	40%	3.627±0.135	4.014±0.239	11.393	11.558
6	50%	7.036±0.355	7.917±0.650	22.103	22.798
7	60%	17.822±1.407	20.158±1.960	55.987	58.047
8	70%	48.532±5.189	57.447±8.263	152.464	165.424
9	80%	98.331±9.492	111.315±10.107	308.906	320.543
10	90%	141.469±10.415	157.318±11.029	444.425	453.014
11	100%	204.589±14.103	225.891±17.485	642.715	650.477

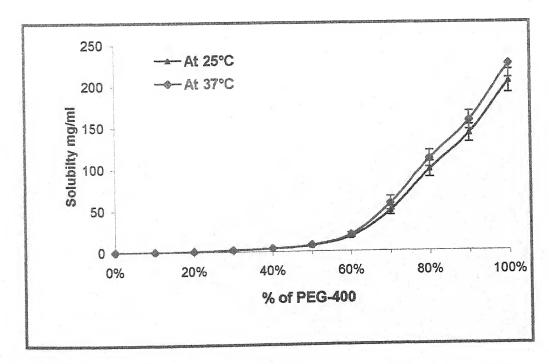


Fig. 4.3.2: Solubility plot of chlorzoxazone in PEG-400

Table 4.3.3: Solubility of chlorzoxazone in various concentration of propylene glycol

S.	Conc. of	Solubility	in mg/ml	Enhancem	ent Factor
No.	PG	At 25°C	At 37°C	At 25°C	At 37°C
1	0%	0.318±0.013	0.347±0.012	1.000	1.000
2	10%	0.443±0.018	0.509±0.015	1.393	1.467
3	20%	0.514±0.016	0.577±0.018	1.613	1.662
4	30%	0.813±0.024	0.913±0.044	2.554	2.628
5	40%	1.292±0.043	1.500±0.058	4.058	4.319
6	50%	2.830±0.080	3.071±0.073	8.890	8.842
7	60%	4.409±0.459	5.270±0.505	13.852	15.176
8	70%	8.877±0.367	9.651±0.536	27.887	27.790
9	80%	14.136±1.110	15.903±1.494	44.407	45.795
10	90%	21.437±1.033	23.867±1.263	67.343	68.728
11	100%	33.118±1.417	37.251±1.763	104.039	107.268

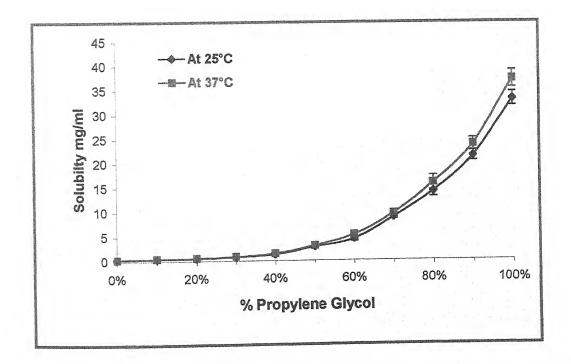


Fig. 4.3.3: Solubility plot of chlorzoxazone in propylene glycol

Table 4.3.4: Solubility of chlorzoxazone in various concentration of ethanol

S.	Conc. of	Solubility	in mg/ml	Enhancem	Enhancement Factor	
No.	ethanol	At 25°C	At 37°C	At 25°C	At 37°C	
1	0%	0.318±0.013	0.347±0.012	1.000	1.000	
2	10%	0.425±0.043	0.456±0.055	1.335	1.312	
3	20%	0.754±0.047	0.857±0.064	2.370	2.468	
4	30%	2.091±0.113	2.346±0.141	6.569	6.756	
5	40%	4.992±0.094	5.495±0.196	15.682	15.822	
6	50%	12.561±0.498	13.739±0.499	39.459	39.563	
7	60%	25.674±1.041	28.648±1.041	80.656	82.495	
8	70%	35.947±2.133	40.097±1.454	112.928	115.464	
9	80%	53.981±3.517	59.428±2.748	169.582	171.129	
10	90%	62.281±2.551	69.589±2.551	195.656	200.389	
11	100%	72.221±4.131	80.617±3.670	226.880	232.145	

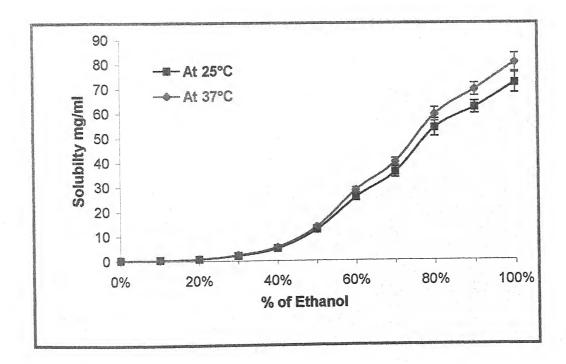


Fig. 4.3.4: Solubility plot of chlorzoxazone in ethanol

Table 4.3.5: Solubility of chlorzoxazone in various concentration of glycerin

S.	Conc. of	Solubility	in mg/ml	Enhancem	ent Factor
No.	glycerin	At 25°C	At 37°C	At 25°C	At 37°C
1	0%	0.318±0.013	0.347±0.012	1.000	1.000
2	10%	0.362±0.015	0.396±0.016	1.137	1.140
3	20%	0.445±0.018	0.489±0.020	1.399	1.408
4	30%	0.532±0.023	0.611±0.026	1.671	1.759
5	40%	0.707±0.028	0.784±0.024	2.220	2.258
6	50%	0.859±0.026	0.946±0.028	2.700	2.724
7	60%	1.058±0.043	1.160±0.061	3.323	3.341
8	70%	1.279±0.060	1.400±0.057	4.018	4.031
9	80%	1.457±0.066	1.657±0.072	4.577	4.772
10	90%	1.695±0.086	1.967±0.098	5.326	5.665
11	100%	2.028±0.145	2.345±0.160	6.371	6.753

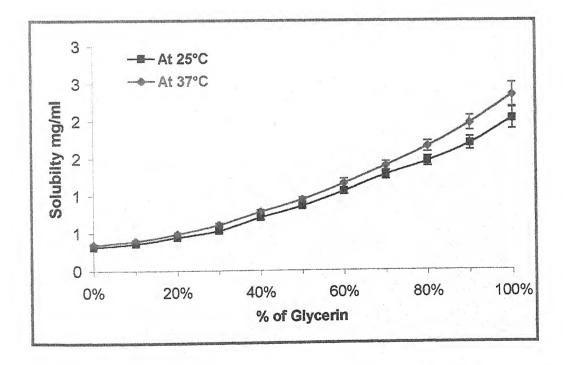


Fig. 4.3.5: Solubility plot of chlorzoxazone in glycerin

Table 4.3.6: Solubility of chlorzoxazone in various proportion of benzyl alcohol

	Conc. of		Solubility in mg/ml					Enhancement Ratio	
S. No.	Benzyl Alcohol	At	25	°C	At	37°	С	At 25°C	At 37°C
1	0%	0.318	<u>±</u>	0.013	0.347	±	0.012	1.000	1.091
2	1%	0.364	±	0.037	0.378	土	0.044	1.142	1.188
3	2%	0.400	±	0.045	0.410	土	0.041	1.258	1.289
4	3%	0.463	土	0.033	0.494	±	0.039	1.453	1.552
5	4%	0.529	±	0.055	0.540	±	0.047	1.661	1.697
6	5%	0.472	±	0.051	0.454	土	0.049	1.482	1.427

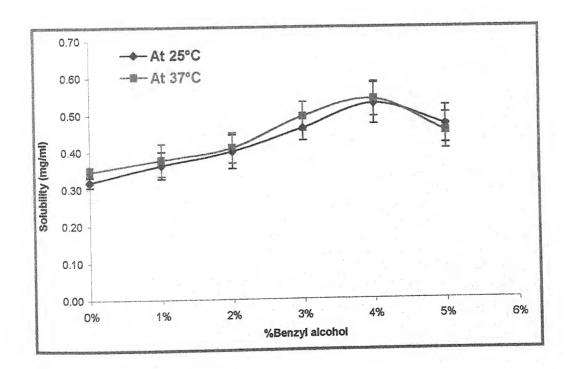


Fig. 4.3.6: Solubility plot of chlorzoxazone in benzyl alcohol

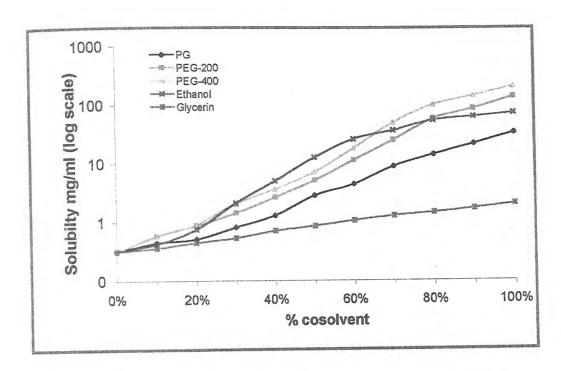


Fig. 4.3.7: Semi-logrithmic solubility plot of chlorzoxazone in different cosolvents at 25°C

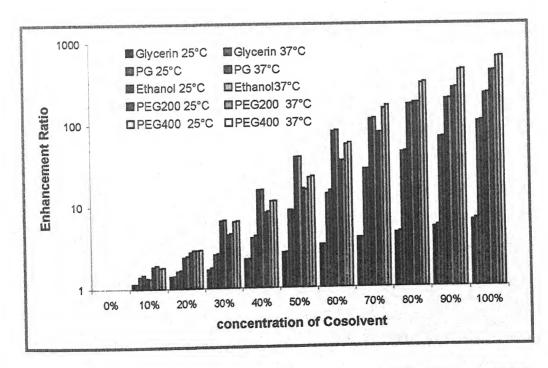


Fig. 4.3.8: Solubility enhancement plot (log scale) of chlorzoxazone in different cosolvents at 25°C

Table 4.3.7: Solubility of chlorzoxazone in various proportion of ethanol-PEG-200

S.	Proportion of	Solubility	in mg/ml	Enhancement Ratio		
No.	cosolvents Ethanol:PEG200	At 25°C	At 37°C	At 25°C	At 37°C	
1	10:0	72.221±4.131	80.617±3.670	226.880	232.145	
2	9:1	70.376±5.054	92.663±5.976	221.086	266.832	
3	8:2	75.448±6.437	94.661±5.207	237.020	272.585	
4	7:3	78.215±8.128	112.797±8.742	245.711	324.812	
5	6:4	97.274±10.126	138.158±11.970	305.584	397.840	
6	5:5	104.344±8.742	131.549±10.587	327.795	378.808	
7	4:6	127.706±5.976	144.613±11.202	401.188	416.429	
8	3:7	136.467±6.744	161.674±7.359	428.710	465.557	
9	2:8	146.304±7.052	171.664±8.281	459.613	494.325	
10	1:9	153.220±6.591	177.966±8.589	481.341	512.472	
11	0:10	136.756±14.411	150.896±14.718	429.618	434.522	

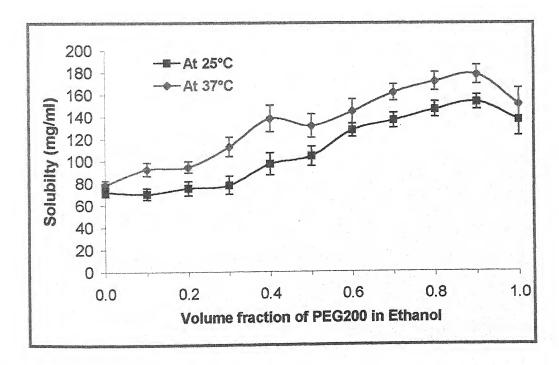


Fig. 4.3.9: Solubility plot of chlorzoxazone in various proportion of Ethanol:PEG200

Table 4.3.8: Solubility of chlorzoxazone in various proportion of ethanol-PEG-400

S.	Proportion of	Solubility	in mg/ml	Enhancen	nent Ratio
No.	cosolvents Ethanol:PEG400	At 25°C	At 37°C	At 25°C	At 37°C
1	10:0	72.221±4.131	80.617±3.670	226.880	232.145
2	9:1	77.572±14.940	72.509±10.107	243.693	208.798
3	8:2	85.257±13.018	96.487±12.874	267.835	277.843
4	7:3	91.405±16.092	111.242±16.255	287.149	320.332
5	6:4	129.062±13.018	133.375±20.251	405.447	384.066
6	5:5	120.636±10.587	129.686±17.485	378.977	373.444
7	4:6	149.811±11.097	157.352±11.952	470.631	453.111
8	3:7	168.209±19.889	201.617±21.174	528.427	580.578
9	2:8	165.566±20.703	190.551±14.103	520.123	548.711
10	1:9	159.830±8.589	192.088±13.181	502.103	553.137
11	0:10	204.589±14.103	225.891±17.485	642.715	650.477

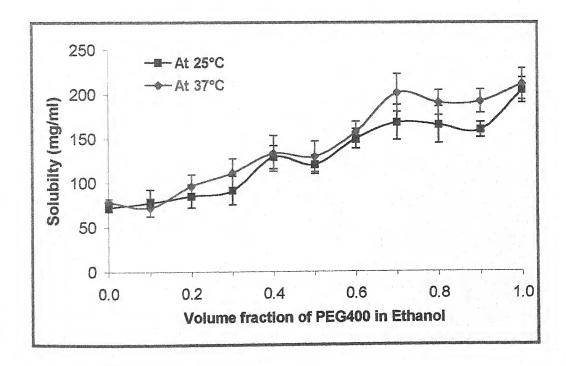


Fig. 4.3.10: Solubility plot of chlorzoxazone in various proportion of Ethanol:PEG400

Table 4.3.9: Solubility of chlorzoxazone in various proportion of ethanol-PG

6	Proportion of	Solubility	in mg/ml	Enhancement Ratio	
S. No.	cosolvents Ethanol:PG	At 25°C	At 37°C	At 25°C	At 37°C
1	10:0	72.221±4.131	80.617±3.670	226.880	232.145
2	9:1	76.035±6.102	82.952±6.486	238.864	238.868
3	8:2	65.661±5.333	69.119±6.102	206.272	199.035
4	7:3	60.665±4.949	67.966±5.333	190.579	195.715
5	6:4	61.050±4.565	65.276±8.023	191.787	187.970
6	5:5	57.447±4.881	65.747±5.189	180.469	189.324
7	4:6	51.164±4.439	52.393±3.978	160.730	150.872
8	3:7	48.243±3.978	48.704±3.670	151.556	140.249
9	2:8	45.784±3.517	46.553±3.517	143.830	134.053
10	1:9	46.245±3.670	50.549±4.439	145.279	145.561
11	0:10	33.118±1.417	37.251±1.763	104.039	107.268

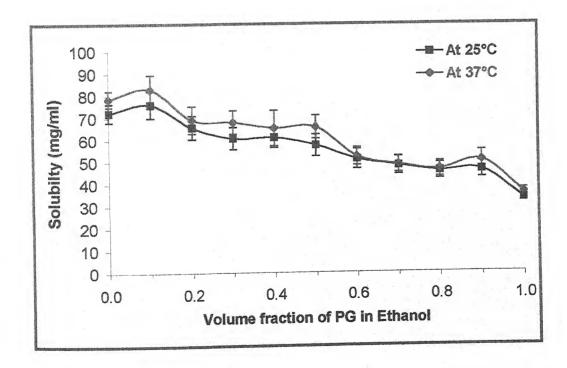


Fig. 4.3.11: Solubility plot of chlorzoxazone in various proportion of Ethanol:PG

S.	Proportion of	Solubility	in mg/ml	Enhancen	nent Ratio
No.	cosolvents PEG200:PEG400	At 25°C	At 37°C	At 25°C	At 37°C
1	10:0	136.756±14.411	150.896±14.718	429.618	434.522
2	9:1	145.997±7.513	156.909±6.591	458.647	451.836
3	8:2	157.370±7.974	169.359±8.742	494.378	487.687
4	7:3	158.446±8.896	193.951±11.816	497.758	558.502
5	6:4	180.272±11.202	188.733±10.126	566.322	543.476
6	5:5	163.211±9.050	184.441±9.204	512.726	531.117
7	4:6	148.609±7.820	177.351±6.744	466.855	510.701
8	3:7	150.454±8.281	171.050±10.587	472.650	492.555
9	2:8	164.748±10.279	180.118±11.663	517.554	518.668
10	1:9	182.885±9.050	191.953±13.507	574.530	552.748
11	0:10	204.589±14.103	225.891±17.485	642.715	650.477

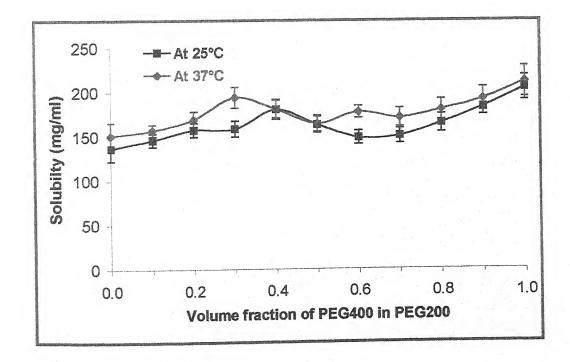


Fig. 4.3.12: Solubility plot of chlorzoxazone in various proportion of PEG200:PEG400

4.3.2 Phase Solubility Study of Indomethacin in Cosolvent System

The equilibrium phase solubility experiment of indomethacin was performed by the same method as used for chlorzoxazone. Solvent systems were prepared as above. An excess quantity of indomethacin was equilibrated with the cosolvent solutions for 72 hrs at 25±2°C and 37±2°C then centrifuged and filtered through 0.45µ membrane syringe filter, The filtrate diluted suitably with distilled water and analyzed spectrophotometrically at 319.5 nm against respective solvent system diluted accordingly as blank. The solubility of Indomethacin was determined in triplicate. Solubility of indomethacin in mg/ml was calculated in different cosolvent solution of different concentration and shown in table 4.3.11-4.3.20 and graphically presented in figure 4.3.13-4.3.18. Comparative semi-log solubility plot in different cosolvent at 25°C is presented in figure 4.3.19.

Solubility enhancement ratios are reported in the same tables and graphically presented in figure 4.3.20. Plots of solubility of indomethacin in cosolvent mix are presented in figures 4.3.21-4.3.24.

4.3.3 Determination of Physico Chemical Parameters of Solvent System *pH*

The pH of saturated drug solution in different cosolvent systems of various proportion was measured at 25±2°C using pH meter. The results are reported in table 4.3.21-4.3.22.

Viscosity

The viscosity of different cosolvent solutions (0 -100%) was determined using water as reference at 25±2°C by Ostwald Viscometer. The viscosity was calculated using following equation¹³.

$$\eta_1 = \frac{\rho_1 t_1}{\rho_2 t_2} \times \eta_2$$

Where, η_1 and η_2 are viscosities, ρ_1 and ρ_2 are densities and t_1 and t_2 are times required for the flow of unknown cosolvent solution and reference liquid, respectively. The values are recorded in table 4.3.23.

Table 4.3.11: Solubility of indomethacin in various concentration of PEG-200

S. No.	Conc. of PEG-200	Solubility in mg/ml		Enhancement Ratio	
		At 25°C	At 37°C	At 25°C	At 37°C
1	0%	0.025±0.005	0.028±0.007	1,000	1.000
2	10%	0.030±0.002	0.039±0.005	1.185	1.379
3	20%	0.036±0.005	0.054±0.005	1.461	1.899
4	30%	0.070±0.008	0.107±0.017	2.812	3.751
5	40%	0.160±0.027	0.180±0.027	6.394	6.338
6	50%	0.314±0.016	0.600±0.064	12.581	21.120
7	60%	1.123±0.032	2.084±0.283	44.986	73.414
8	70%	4.573±0.609	6.390±0.744	183.116	225.039
9	80%	15.000±0.485	16.301±2.411	600.668	574.127
10	90%	33.336±0.886	44.100±2.943	1334.906	1553,202
11	100%	75.188±5.221	85.999±8.412	3010.868	3028,902

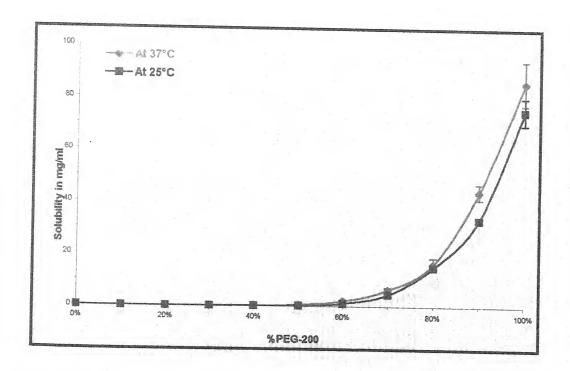


Fig. 4.3.13: Solubility plot of indomethacin in PEG-200

Table 4.3.12: Solubility of indomethacin in various concentration of PEG-400

S. No.	Conc. of PEG-400	Solubility in mg/ml		Enhancement Ratio	
		At 25°C	At 37°C	At 25°C	At 37°C
1	0%	0.025 ± 0.005	0.028 ± 0.007	1.000	1.000
2	10%	0.058 ± 0.013	0.070 ± 0.008	2.306	2.454
3	20%	0.106 ± 0.006	0.134 ± 0.023	4.226	4.703
4	30%	0.228 ± 0.022	0.267 ± 0.038	9.130	9.404
5	40%	0.461 ± 0.035	0.546 ± 0.037	18.461	19.230
6	50%	1.302 ± 0.201	1.527 ± 0.227	52.143	53.781
7	60%	2.811 ± 0.419	3.254 ± 0.554	112.570	114.607
8	70%	7.167 ± 0.664	8.167 ± 0.903	286.999	287.643
9	80%	19.143 ± 0.882	22.578 ± 0.683	766.581	795,202
10	90%	47.353 ± 3.295	54.218 ± 3.408	1896,214	1909.569
11	100%	16.064 ± 11.113	134.108 ± 8.665	4647.718	4723.310

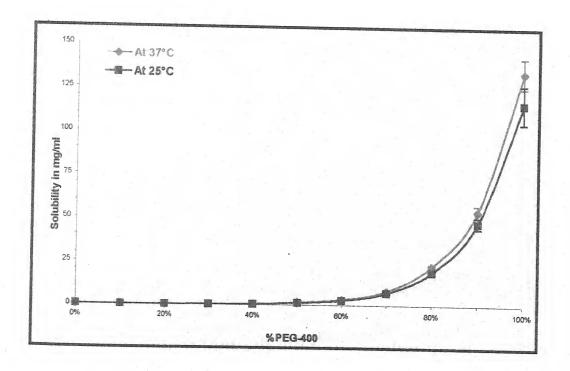


Fig. 4.3.14: Solubility plot of indomethacin in PEG-400

Table 4.3.13: Solubility of indomethacin in various concentration of propylene glycol

S.	Conc. of	Solubility	in mg/ml	Enhancen	Enhancement Ratio		
No.	PG	At 25°C	At 37°C	At 25°C	At 37°C		
1	0%	0.025±0.005	0.028±0.007	1.000	1.000		
2	10%	0.066±0.010	0.097±0.025	2.653	3.423		
3	20%	0.094±0.022	0.165±0.032	3.774	5.827		
4	30%	0.137±0.015	0.185±0.031	5.497	6.530		
5	40%	0.157±0.025	0.169±0.026	6.276	5.970		
6	50%	0.187±0.025	0.339±0.039	7.495	11.953		
7	60%	0.247±0.073	0.360±0.154	9.893	12.693		
8	70%	0.405±0.041	0.494±0.054	16.218	17.391		
9	80%	0.981±0.043	1.187±0.056	39.284	41.815		
10	90%	2.039±0.094	2.851±0.239	81.656	100.409		
11	100%	7.126±0.724	8.206±0.773	285.357	289.024		

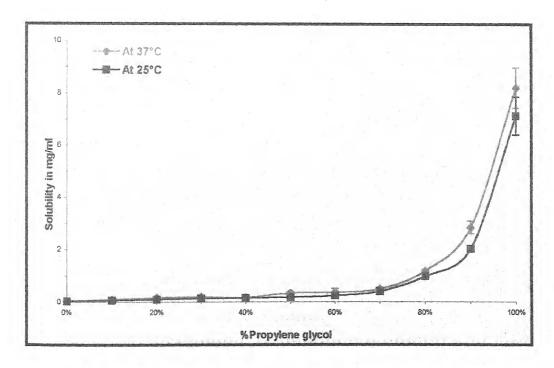


Fig. 4.3.15: Solubility plot of indomethacin in propylene glycol

Table 4.3.14: Solubility of indomethacin in various concentration of ethanol

S.	Conc. of	Solubility	in mg/ml	Enhancer	nent Ratio
No.	Ethanol	At 25°C	At 37°C	At 25°C	At 37°C
1	0%	0.025±0.005	0.028±0.007	1.000	1.000
2	10%	0.075±0.016	0.106±0.016	3.007	3.723
3	20%	0.102±0.025	0.255±0.070	4.095	8.981
4	30%	0.160±0.021	0.614±0.105	6.402	21.625
5	40%	0.234±0.019	1.086±0.197	9.377	38.249
6	50%	0.464±0.050	1.967±0.154	18.597	69.278
7	60%	1.055±0.083	3.125±0.263	42.233	110.063
8	70%	1.930±0.094	4.683±0.475	77.297	164.936
9	80%	3.743±0.309	7.238±1.627	149.875	254.924
10	90%	8.370±1.763	11.261±2.271	335.167	396.615
11	100%	18.755±2.152	23.354±2.358	751.016	822.517

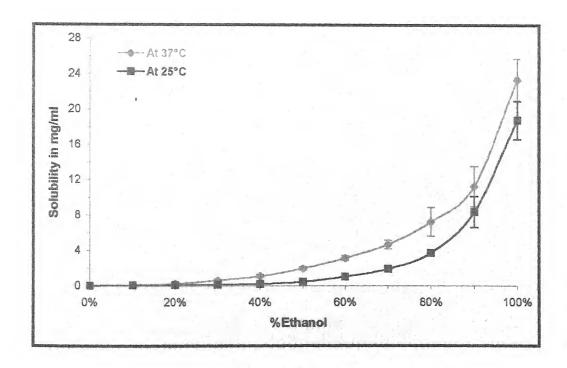


Fig. 4.3.16: Solubility plot of indomethacin in ethanol

Table 4.3.15: Solubility of indomethacin in various concentration of glycerin

S.	Conc. of	Solubility	in mg/ml	Enhancement Ratio		
No.	Glycerine	At 25°C	At 37°C	At 25°C	At 37°C	
1	0%	0.025±0.005	0.028±0.007	1.000	1.000	
2	10%	0.056±0.008	0.068±0.021	2.247	2.391	
3	20%	0.116±0.020	0.137±0.022	4.652	4.825	
4	30%	0.159±0.013	0.189±0.034	6.382	6.657	
5	40%	0.200±0.009	0.244±0.019	8.001	8.580	
6	50%	0.258±0.029	0.312±0.042	10.347	10.989	
7	60%	0.339±0.129	0.417±0.157	13.575	14.687	
8	70%	0.524±0.104	0.615±0.119	20.966	21.660	
9	80%	0.642±0.190	0.726±0.187	25.718	25.570	
10	90%	0.749±0.127	0.874±0.211	30.010	30.782	
11	100%	0.877±0.191	1.049±0.256	35.122	36.946	

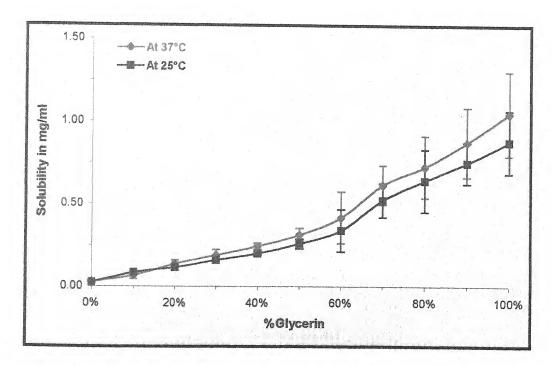


Fig. 4.3.17: Solubility plot of indomethacin in glycerin

Table 4.3.16: Solubility of indomethacin in various proportion of benzyl alcohol

s.	Conc. of Benzyl	Solubility	in mg/ml	Enhancement Ratio	
No.	Alcohol	At 25°C	At 37°C	At 25°C	At 37°C
1	0%	0.025±0.005	0.028±0.007	1.000	1.000
2	0.5%	0.042±0.005	0.051±0.003	1.674	1.805
3	1.0%	0.047±0.004	0.058±0.009	1.865	2.057
4	1.5%	0.050±0.004	0.066±0.011	2.017	2.316
5	2.0%	0.063±0.008	0.071±0.010	2.542	2.506
6	2.5%	0.067±0.008	0.076±0.013	2.686	2.662
7	3.0%	0.070±0.007	0.088±0.011	2.797	3.100
8	3.5%	0.077±0.014	0.095±0.013	3.066	3.336
9	4.0%	0.088±0.014	0.104±0.013	3.525	3.665

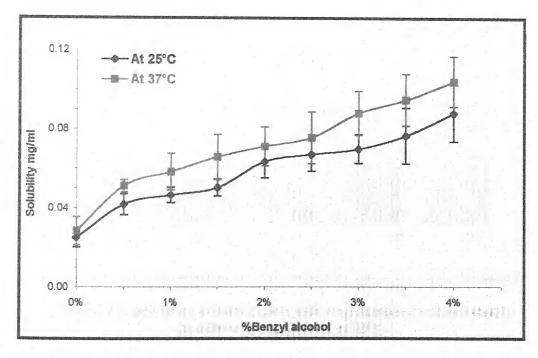


Fig. 4.3.18: Solubility plot of indomethacin in benzyl alcohol

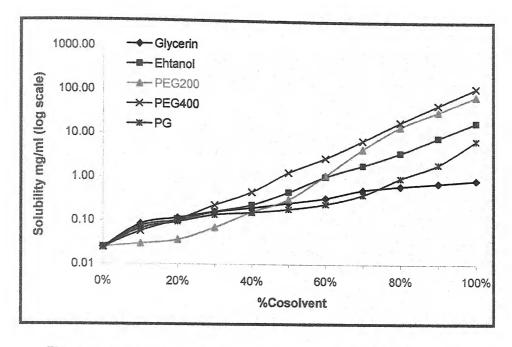


Fig. 4.3.19: Semi-logrithmic Solubility plot of indomethacin in different cosolvents at 25°C

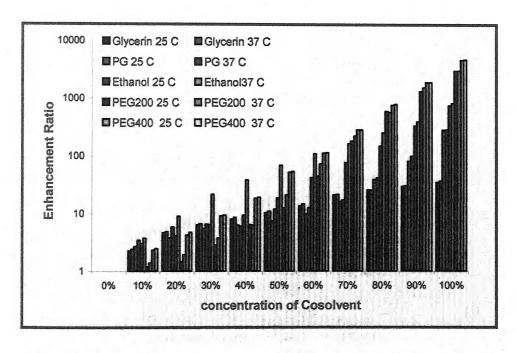


Fig. 4.3.20: Solubility enhancement plot (log scale) of indomethacin in different cosolvents at 25°C

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Table 4.3.17: Solubility of indomethacin in various proportion of ethanol-PEG-200

S.	Proportion of cosolvents	Solubility	in mg/ml	Enhancen	nent Ratio
No.	No. Ethanol:PEG200	At 25°C	At 37°C	At 25°C	At 37°C
1	10:0	18.755±2.152	23.354±2.358	751.016	822.517
2	9:1	33.139±2.740	34.776±1.266	1327.041	1224.811
3	8:2	31.961±4.092	36.854±3.008	1279.853	1298.005
4	7:3	39.571±4.041	57.892±7.495	1584.611	2038.968
5	6:4	53.401±1.751	74.987±9.459	2138.421	2641.057
6	5:5	63.467±8.554	77.319±5.507	2541.506	2723.190
7	4:6	58.926±3.745	75.234±10.712	2359.664	2649.756
8	3:7	59.928±4.697	76.260±9.607	2399.789	2685.901
9	2:8	67.980±4.691	77.447±6.121	2722.243	2727.693
10	1:9	77.997±4.761	86.808±4.915	3123.345	3057.407
11	0:10	75.188±5.221	85.999±8.412	3010.868	3028.902

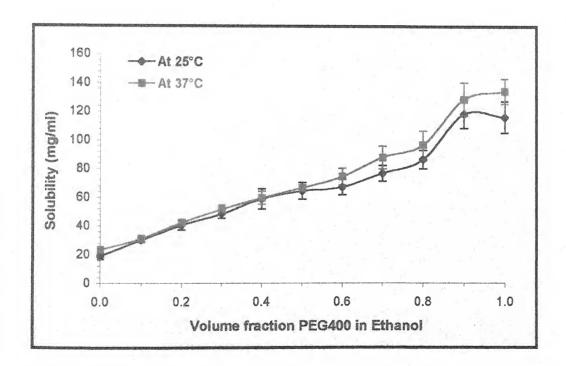


Fig. 4.3.21: Solubility plot of indomethacin in various proportion of Ethanol:PEG400

Table 4.3.18: Solubility of indomethacin in various proportion of ethanol-PEG-400

S.	Proportion of cosolvents	Solubility	in mg/ml	Enhancement Ratio	
No.	Ethanol:PEG400	At 25°C	At 37°C	At 25°C	At 37°C
1	10:0	18.755±2.152	23.354±2.358	751.016	822.517
2	9:1	30.1440±1.535	31.1915±2.218	1207.104	1098.571
3	8:2	40.4550±3.305	42.3045±2.353	1620.003	1489.972
4	7:3	48.3929±2.959	51.5917±3.057	1937.870	1817.070
5	6:4	58.8953±7.024	59.5705±4.524	2358.436	2098.083
6	5:5	64.4395±5.876	66.5468±2.164	2580.451	2343.790
7	4:6	67.2275±5.405	74.4616±5.744	2692.095	2622.552
8	3:7	76.7529±5.478	87.8397±7.925	3073.535	3093.730
9	2:8	86.4894±6.399	96.6368±9.708	3463.428	3403.565
10	1:9	118.3960±10.131	128.561±11.604	4741.112	4527.944
11	0:10	116.064±11.113	134.108±8.665	4647.728	4723.310

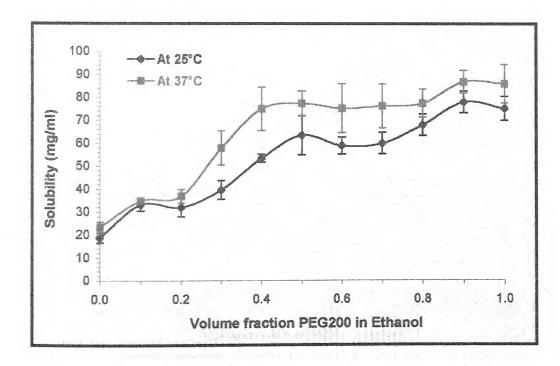


Fig. 4.3.22: Solubility plot of indomethacin in various proportion of Ethanol:PEG200

Table 4.3.19: Solubility of indomethacin in various proportion of ethanol-PG

S.	Proportion of	Solubility	in mg/ml	Enhancer	nent Ratio
No.	cosolvents Ethanol:PG	At 25°C	At 37°C	At 25°C	At 37°C
1	10:0	18.755±2.152	23.354±2.358	751.016	822.517
2	9:1	16.823±0.916	21.308±1.876	673.679	750.462
3	8:2	15.588±0.620	19.262±1.476	624.197	678.407
4	7:3	14.589±1.607	17.797±2.021	584.218	626.816
5	6:4	13.755±2.129	17.891±1.751	550.792	630.125
6	5:5	13.869±1.008	16.390±4.473	555.380	577.243
7	4:6	13.133±2.110	14.229±1.442	525.887	501.153
8	3:7	11.897±2.203	12.537±1.295	476.405	441.556
9	2:8	10.203±1.100	11.283±1.620	408.572	397.394
10	1:9	8.607±0.917	9.646±1.648	344.671	339.750
11	0:10	7.126±0.724	8.206±0.773	285.357	289.024

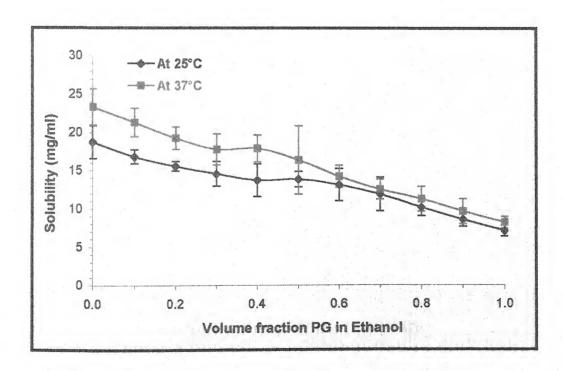


Fig. 4.3.23: Solubility plot of indomethacin in various proportion of Ethanol:PG

Table 4.3.20: Solubility of indomethacin in various proportion of PEG-400-PEG-200

S.	Proportion of cosolvents	Solubility	in mg/ml	Enhancement Ratio	
No.	PEG400:PEG200	At 25°C	At 37°C	At 25°C	At 37°C
1	10:0	116.064±11.113	134.108±8.665	4647.728	4723.310
2	9:1	118.478±9.947	127.315±0.933	4744.389	4484.059
3	8:2	106.530±9.149	109.722±9.640	4265.950	3864.419
4	7:3	96.956±6.939	108.331±7.921	3882.544	3815.422
5	6:4	99.276±14.795	107.649±13.077	3975.461	3791.419
6	5:5	95.673±13.568	109.558±10.131	3831.180	3858.654
7	4:6	96.547±6.939	102.684±21.669	3866.159	3616.551
8	3:7	87.790±7.676	97.038±10.622	3515.523	3417.680
9	2:8	82.471±9.394	90.573±12.831	3302.519	3189.987
10	1:9	79.443±10.376	82.880±11.113	3181.271	2919.062
11	0:10	75.188±5.221	85.999±8.412	3010.868	3028.902

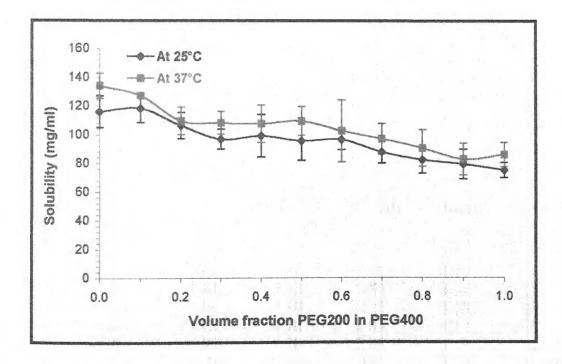


Fig. 4.3.24: Solubility plot of indomethacin in various proportion of PEG400:PEG200

Table 4.3.21: pH of saturated solution of chlorzoxazone in cosolvent-water mixture

S. No.	Conc. Of cosolvent	PG	PEG-200	PEG-400	Ethanol	Glycerin
1.	0%	6.82	6.82	6.82	6.82	6.00
2.	10%	6.80	6.78	6.79	6.78	6.82
3.	20%	6.64	6.74	6.76	6.74	6.83
4.	30%	6.41	6.72	6.73	6.89	6.84
5.	40%	6.37	6.70	6.69	6.91	6.81
6	50%	6.32	6.67	6.67	6.92	6.79
7.	60%	6.29	6.63	6.66	6.92 6.96	6.78
8.	70%	6.28	6.58	6.63		6.75
9.	80%	6.28	6.55	6.63	6.98	6.73
10.	90%	6.26	6.5		7.01	6.73
11.	100%	6.23		6.62	7.01	6.71
11.	10070	0.23	6.44	6.63	7.03	6.71

Table 4.3.22: pH of saturated solution of indomethacin in cosolvent-water mixture

S. No.	Conc. Of cosolvent	PG	PEG-200	PEG-400	Ethanol	Glycerin
1.	0%	6.73	6.73	6.73	6.73	6.73
2.	10%	6.64	6.51	6.69	6.68	6.65
3.	20%	6.41	6.39	6.53	6.55	6.63
4.	30%	6.32	6.32	6.46	6.52	6.61
5.	40%	6.26	6.18	6.46	6.49	6.59
6	50%	6.16	6.02	6.45	6.43	6.41
7.	60%	6.02	6.00	6.42	6.38	6.29
8.	70%	6.00	5.91	6.41	6.29	6.25
9.	80%	5.81	5.86	6.38	6.23	6.18
10.	90%	5.70	5.61	6.21	6.19	6.11
11.	100%	5.35	5.28	5.98	5.73	6.03

Table 4.3.23: Viscosity (cps.)of cosolvent-water mixture

S. No.	Conc. Of cosolvent	PG	PEG-200	PEG-400	Ethanol	Glycerin
1.	0%	1.00	1.00	1.00	1.00	1.00
2.	10%	1.24	1.22	1.29	1.02	1.32
3.	20%	1.68	1.60	1.72	1.08	1.98
4.	30%	2.21	2.12	2.51	1.07	3.31
5.	40%	3.08	2.99	3.23	1.08	5.01
6	50%	4.72	4.50	5.42	1.11	7.41
7.	60%	6.88	6.38	7.66	1.11	13.18
8.	70%	10.46	9.82	11.73	1.13	25.46
9.	80%	17.24	16.26	18.94	1.18	104.71
10.	90%	25.98	22.55	30.54	1.18	
11.	100%	58.10	45.00	103.30	1.22	

Surface Tension

The surface tension of different cosolvent solutions (0 -100%)) was determined using water as references at 25±2°C using Stalagmometer. The surface tension was calculated using following equation 13.

$$\gamma_1 = \frac{\rho_1 n_1}{\rho_2 n_2} \times \gamma_2$$

Where, γ_1 and γ_2 are surface tension, ρ_1 and ρ_2 are densities and n_1 and n_2 are number of drops formed of unknown cosolvent solution and reference liquid water respectively. The values are recorded in table 4.3.24.

Dielectric constant

Dielectric constant of the solvent mixtures was calculated from the relation^{8,14} $M_{hix} = M_{vs} f_{ws} + M_{s} f_{ss}$,

where M and f are the dielectric constant and volume fraction, respectively; and subscripts mix, ws, and ss represent values for the mixture, weaker solvent, and stronger solvent, respectively. These values are provided in table 4.3.25.

Solubility parameter¹⁵

In 1936 Joel H. Hildebrand (who laid the foundation for solubility theory in his classic work on the solubility of nonelectrolytes in 1916) proposed the square root of the cohesive energy density as a numerical value indicating the solvency behavior of a specific solvent.

$$\delta = \sqrt{C} = \left[\frac{\Delta H - RT}{V_m} \right]^{1/2}$$

The term "solubility parameter" was proposed for this value and the quantity represented by a delta (δ).

It is an interesting aspect of the Hildebrand solvent spectrum that the Hildebrand value of a solvent mixture can be determined by averaging the Hildebrand values of the individual solvents by volume. Solubility parameter of the solvent mixtures were calculated from the relation

$$\delta_{\text{mix}} = \delta_1 f + \delta_2 (1 - f)$$

The values are recorded in table 4.3.26.

Table 4.3.24: Surface tension (dynes/cm) of cosolvent-water mixture

S. No.	Conc. Of cosolvent	PG	PEG-200	PEG-400	Ethanol	Glycerin
1.	0%	71.61	71.61	71.61	71.61	71.61
2.	10%	68.80	65.16	66.55	56.91	67.55
3.	20%	65.59	60.88	62.08	47.42	66.60
4.	30%	62.66	55.72	57.27	41.64	65.73
5.	40%	58.48	52.48	53.53	35.82	65.49
6	50%	54.83	49.67	50.95	30.31	64.71
7.	60%	47.74	48.52	49.58	28.14	64.52
8.	70%	44.42	47.63	48.28	25.87	64.33
9.	80%	41.53	46.12	46.46	24.16	63.65
10.	90%	40.10	44.85	45.60	23.28	63.49
11.	100%	35.80	43.79	44.23	22.81	63.38

Table 4.3.25: Dielectric constant of cosolvent-water mixture

S. No.	Conc. Of cosolvent	PG	PEG-200	PEG-400	Ethanol	Glycerin
1.	0%	78.5	78.5	78.5	78.5	78.5
2.	10%	73.85	72.01	71.9	73.08	74.9
3.	20%	69.2	65.52	65.3	67.66	71.3
4.	30%	64.55	59.03	58.7	62.24	67.7
5.	40%	59.9	52.54	52.1	56.82	64.1
6	50%	55.25	46.05	45.5	51.4	60.5
7.	60%	50.6	39.56	38.9	45.98	56.9
8.	70%	45.95	33.07	32.3	40.56	53.3
9.	80%	41.3	26.58	25.7	35.14	49.7
10.	90%	36.65	20.09	19.1	29.72	46.1
11.	100%	32	13.6	12.5	24.3	42.5

Table 4.3.26: Dielectric constant of cosolvent-cosolvent mixture

S. No.	Conc. Of cosolvent	Ethanol:PG	Ethanol: PEG-200	Ethanol: PEG-400	PEG200: PEG400
1.	0%	32.00	24.30	24.30	13.60
2.	10%	31.23	23.23	23.12	13.49
3.	20%	30.46	22.16	21.94	13.38
4.	30%	29.69	21.09	20.76	13.27
5.	40%	28.92	20.02	19.58	13.16
6	50%	28.15	18.95	18.40	13.05
7.	60%	27.38	17.88	17.22	12.94
8.	70%	26.61	16.81	16.04	12.83
9.	80%	25.84	15.74	14.86	12.72
10.	90%	25.07	14.67	13.68	12.61
11.	100%	24.30	13.60	12.50	12.50

Table 4.3.27: Solubility parameter in (cal/cm³)^{0.5} of cosolvent-water mixture

Conc. of cosolvent	PG	PEG-200	PEG-400	Ethanol	Glycerin
0%	23.40	23.40	23.70	23.40	23.40
10%	22.32	22.28	22.46	22.33	22.83
20%	21.24	21.16	21.22	21.26	22.26
30%	20.16	20.04	19.98	20.19	21.69
40%	19.08	18.92	18.74	19.12	21.12
50%	18.00	17.80	17.50	18.05	20.55
60%	16.92	16.68	16.26	16.98	19.98
70%	15.84	15.56	15.02	15.91	19.41
80%	14.76	14.44	13.78	14.84	18.84
90%	13.68	13.32	12.54	13.77	18.27
100%	12.60	12.20	11.30	12.70	17.70
	0% 10% 20% 30% 40% 50% 60% 70% 80%	cosolvent PG 0% 23.40 10% 22.32 20% 21.24 30% 20.16 40% 19.08 50% 18.00 60% 16.92 70% 15.84 80% 14.76 90% 13.68	Cosolvent PG PEG-200 0% 23.40 23.40 10% 22.32 22.28 20% 21.24 21.16 30% 20.16 20.04 40% 19.08 18.92 50% 18.00 17.80 60% 16.92 16.68 70% 15.84 15.56 80% 14.76 14.44 90% 13.68 13.32	Cosolvent PG PEG-200 PEG-400 0% 23.40 23.40 23.70 10% 22.32 22.28 22.46 20% 21.24 21.16 21.22 30% 20.16 20.04 19.98 40% 19.08 18.92 18.74 50% 18.00 17.80 17.50 60% 16.92 16.68 16.26 70% 15.84 15.56 15.02 80% 14.76 14.44 13.78 90% 13.68 13.32 12.54	Cosolvent PG PEG-200 PEG-400 Ethanol 0% 23.40 23.40 23.70 23.40 10% 22.32 22.28 22.46 22.33 20% 21.24 21.16 21.22 21.26 30% 20.16 20.04 19.98 20.19 40% 19.08 18.92 18.74 19.12 50% 18.00 17.80 17.50 18.05 60% 16.92 16.68 16.26 16.98 70% 15.84 15.56 15.02 15.91 80% 14.76 14.44 13.78 14.84 90% 13.68 13.32 12.54 13.77

Table 4.3.28: Partition coefficient (log P) of drug and solvent system

Drug/Solvent	Partition Coefficient (log <i>P</i>)
Chlorzoxazone	1.938±0.296
Indomethacin	3.172±0.258
Propylene Glycol	-0.193
PEG - 200	-
PEG - 400	
Ethanol	0.133
Glycerin	-1.223
	Chlorzoxazone Indomethacin Propylene Glycol PEG - 200 PEG - 400 Ethanol

4.3.4 Mathematical Analysis of Phase Solubility Data

The log-linear model¹⁶ describes an exponential increase in a non-polar drugs solubility with a linear increase in cosolvent concentration. This relationship is described algebraically by¹⁶⁻¹⁸:

$$log S_{mix} = log S_W + \sigma f_C, \qquad ...(1)$$

Where S_{mix} and S_W are the total solute solubilities in the cosolvent-water mixture and in water, respectively, σ is the cosolvent solubilization power for the particular cosolvent-solute system, and f_C is the volume fraction of the cosolvent in the aqueous mixture. Thus to determine the degree of solubilization of a certain compound by a particular cosolvent, one needs a value for the solubilization power term, σ . One way to obtain this value is by experimentation where individual 'sigma' (σ) terms can be obtained from the slope of the log (S_{mix}/S_W) vs. cosolvent volume fraction (f_C) profile of each selected drug cosolvent.

The log-linear model's predictive ability and the focus of this relationship exists between σ and the logrithm of the solute's partition coefficient (log K_{OW})¹⁹. This is a key relationship and critical to appreciate. Essentially it describes a linear correlation between how strongly a solute is solubilized to how hydrophobic the compound is. In essence, the more hydrophobic the solute, the more it will be solubilized by cosolvent addition. This linear relationship for solubilization power can be algebraically described by the following simple formula of a line:

$$\sigma = s \log K_{OW} + t, \qquad \dots (2)$$

Where 's' and 't' are cosolvent constants that are solute independent and $\log K_{OW}$ is the partition coefficient of the solute of interest. The parameters s and t are the linear regression terms for slope and intercept, respectively, obtained from data sets of solubilization power versus solute polarity for each cosolvent. An expanded form of the log-linear equation is obtained by substituting σ from Eq. (2) into Eq. (1) giving:

$$\log S_{mix} = \log S_W + (s \log K_{OW} + t)f_c, \qquad(3)$$

The log-linear model has been shown to work for mixed cosolvent system in the following form:

$$\log S_{\text{mix}} = \log S_{\text{W}} + \Sigma(\sigma f_{\text{c}}) \qquad ...(4)$$

Where the individual solubilization powers and volume fractions for each cosolvent are linearly summed. This is assuming that there are no specific non-ideal interactions between the different cosolvents.

Table 4.3.29: Solubilizing power of various cosolvents for chlorzoxazone

Cosolvent	Solubilizin	g power (ठ)		,2
Cosoiveiit	At 25°C	At 37°C	At 25°C	At 37°C
PEG-400	2.9125	2.9317	0.9897	0.989
PEG-200	2.6381	2.6444	0.9898	0.9906
Ethanol	2.6991	2.7104	0.954	0.9545
Propylene Glycol	1.9671	1.986	0.9766	0.9801
Glycerin	0.8255	0.8459	0.9943	0.997

Table 4.3.30: Stability constant for Chlorzoxazone Cosolvent interaction

S.No.	Cosolvent	Temperature (°C)	Solubility in Water (mM)	К
1	1 PEG-200	25±2	1.88	429.6
ı		37±2	2.05	434.5
2	PEG-400	25±2	1.88	642.7
2		37±2	2.05	650,5
3	Dranylana Clysol	25±2	1.88	104
3	Propylene Glycol	37±2	2.05	107.3
1	[thanal	25±2	1.88	226.9
4	4 Ethanol	37±2	2.05	232.1
	Chrosin	25±2	1.88	6.371
5	Glycerin	37±2	2.05	6.753

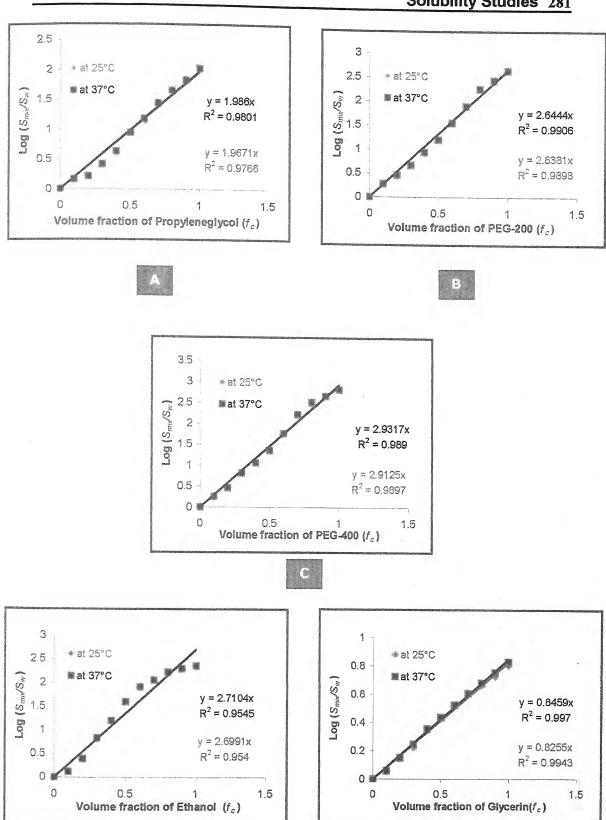


Fig. 4.3.25: Plots for determination of solubilizing power of (A) Prpylene Glycol (B) PEG-200 (C) PEG-400 (D) Ethanol (E) Glycerin for Chlorzoxazone

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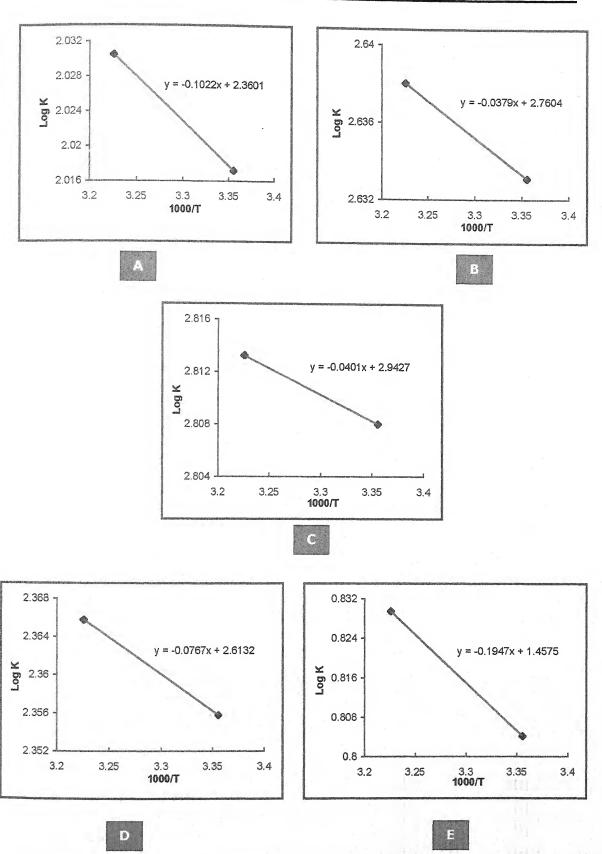


Fig. 4.3.26: Van't Hoff Plots of stability constants for chlorzoxazone-cosolvent (A) Prpylene Glycol (B) PEG-200 (C) PEG- 400 (D) Ethanol (E) Glycerin interactions

Table 4.3.31: Thermodynamic parameter for chlorzoxazone cosolvent interaction

Cosolvent	Temp. (°C)	Stability Constant	ΔG (kJ.mol ⁻¹)	ΔH (kJ.mol ⁻¹)	ΔS (J.mol ⁻¹ .K ⁻¹)
PEG-200	25	429.6	-15.02	1.957	56.974
	37	434.5	-15.05	1.957	54.859
PEG-400	25	642.7	-16.02	0.726	56.192
	37	650.5	-16.05	0.726	54.113
Propylene Glycol	25	104	-11.51	0.768	41.193
	37	107.3	-11.58	0.768	39.843
Ethanol	25	226.9	-13.44	1.469	50.028
	37	232.1	-13.50	1.469	48.275
Glycerin	25	6.371	-4.59	3.728	27.906
	37	6.753	-4.73	3.728	27.291

Table 4.3.32: Solubilizing power of various cosolvents for indomethacin

Cosolvent -	Solubilizin	g power (σ)	r	2
Cosolvent	At 25°C	At 37°C	At 25°C	At 37°C
Propylene Glycol	2.0862	2.1792	0.9221	0.9031
PEG-200	3.1526	3.2602	0.9233	0.9485
PEG-400	3.5561	3.5711	0.9950	0.9958
Ethanol	2.7589	3.1578	0.9888	0.9201
Glycerin	1.8092	1.8282	0.8005	0.8391

Table 4.3.33: Stability constant for indomethacin Cosolvent interaction

S.No.	Cosolvent	Temperature (°C)	Solubility in Water (mM)	К
1	PEG-200	25±2	0.070	3011
	FEG-200	37±2	0.079	3029
2 PEG-400	PEG-400	25±2	0.070	4648
	FEG-400	37±2	0.079	4723
3	Propylene Glycol	25±2	0.070	285.4
	Propylene Glycol	37±2	0.079	289
4	Ethonal	25±2	0.070	751
4	Ethanol	37±2	0.079	822.5
5	Chronin	25±2	0.070	35.12
	Glycerin	37±2	0.079	36.95
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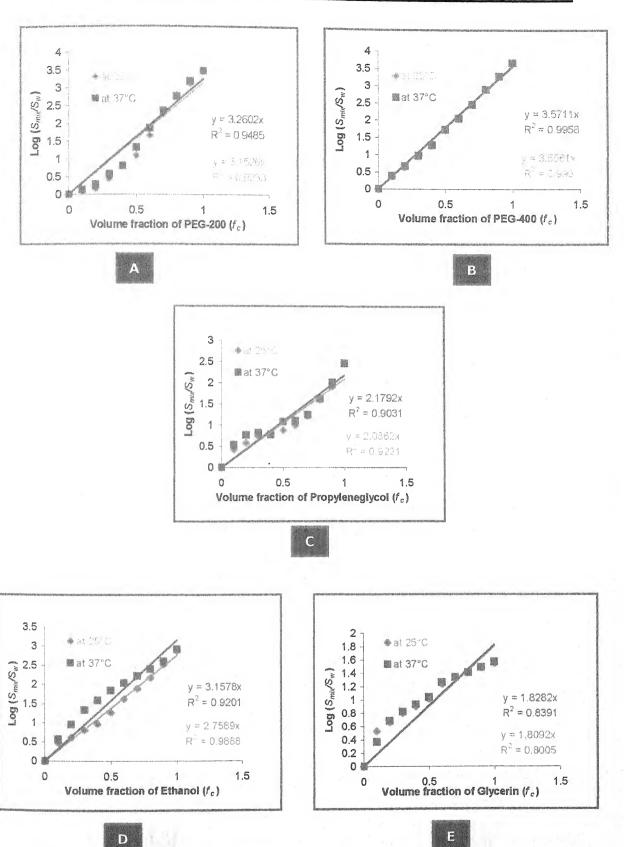


Fig. 4.3.27: Plots for determination of solubilizing power of (A) PEG-200 (B) PEG-400 (C) Prpylene Glycol (D) Ethanol (E) Glycerin for Indomethacin

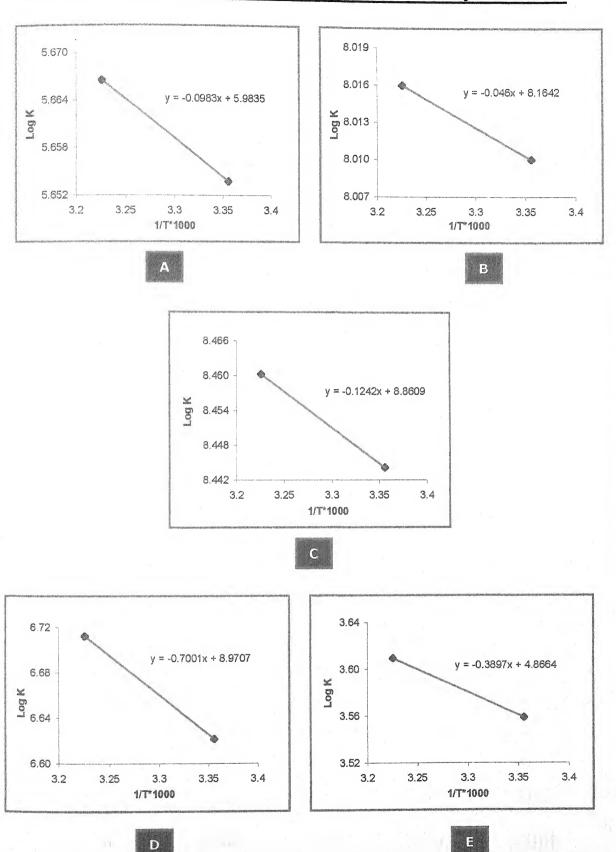


Fig. 4.3.28: Van't Hoff Plots of stability constants for indomethacin-cosolvent (A) PEG-200 (B) PEG- 400 (C) Prpylene Glycol (D) Ethanol (E) Glycerin interactions

Table 4.3.34: Thermodynamic parameter for indomethacin cosolvent interaction

Cosolvent	Temp. (°C)	Stability Constant	∆G (kJ.mol ⁻¹)	ΔH (kJ.mol ⁻¹)	ΔS (J.mol ⁻¹ .K ⁻¹)
PEG-200	25	3011	-19.85	0.881	69.551
	37	3029	-19.86	0.881	66.906
PEG-400	25	4648	-20.92	2.378	78.185
	37	4723	-20.96	2.378	75.287
Propylene Glycol	25	285.4	-14.01	1.882	53.321
	37	289	-14.04	1.882	51.359
Ethanol	25	751	-16.41	13.41	100.04
	37	822.5	-16.63	13.41	96.89
Glycerin	25	35.12	-8.817	7.462	54.628
	37	36.95	-8.943	7.462	52.918

Table 4.3.35: Solubilizing Power of Stronger Cosolvent

	Solubilizing power $(\underline{\sigma})$					
Cosolvent Mix	Chlorzoxazone		Indomethacin			
	At 25°C	At 37°C	At 25°C	At 37°C		
Ethanol/PEG-400	0.4524	0.4913	0.7075	0.7174		
Ethanol/PEG-200	0.3820	0.3275	0.5353	0.5117		
Ethanol/Propylene Glycol	0.3002	0.3124	0.3650	0.4317		
PEG-200/PEG-400	0.1088	0.1064	0.1881	0.1888		

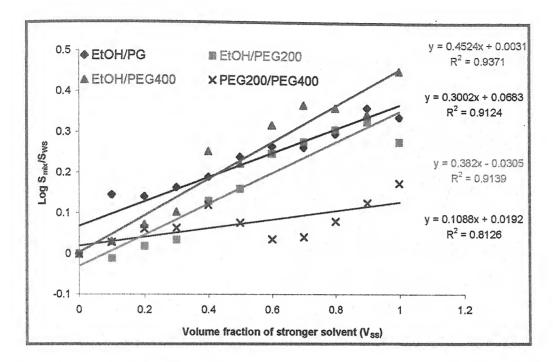


Fig. 4.3.29: Plot of Log S_{mix}/S_{WS} Vs. V_{SS} for chlorzoxazone in cosolvent mixture at 25°C

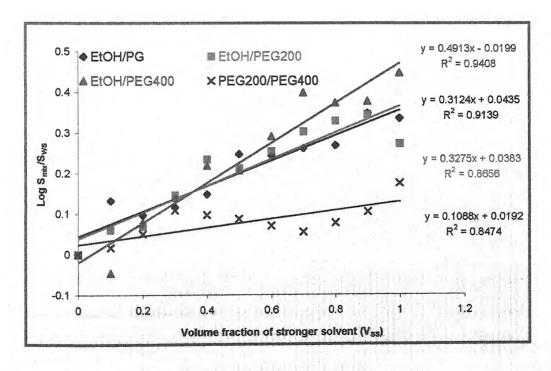


Fig. 4.3.30: Plot of Log S_{mix}/S_{WS} Vs. V_{SS} for chlorzoxazone in cosolvent mixture at 25°C

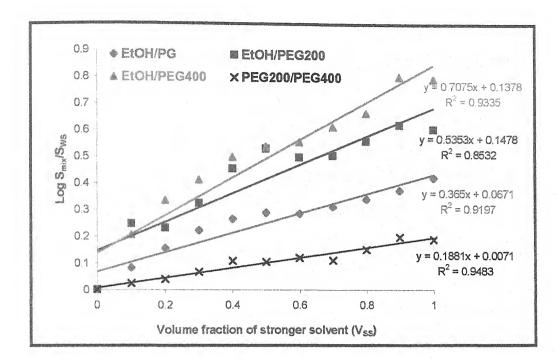


Fig. 4.3.31: Plot for Log S_{mix}/S_{WS} Vs. V_{SS} for indomethacin in different cosolvent mixture at 25°C

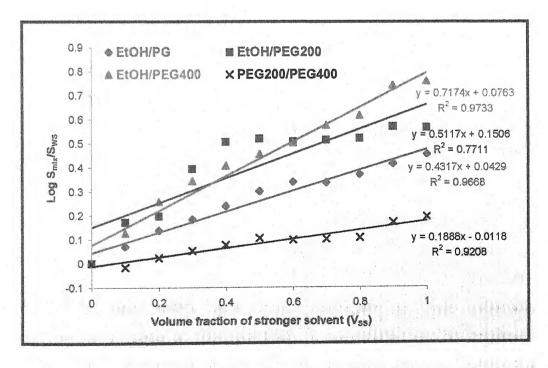


Fig. 4.3.32: Plot for Log S_{mix}/S_{Ws} Vs. V_{ss} for indomethacin in different cosolvent mixture at 37°C

4.3.5 Results and Discussion

Poor solubility is a common concern in the pharmaceutical science. There is several established method for increasing the equilibrium solubility of non-polar drugs in aqueous vehicles. Cosolvency, the addition of water miscible solvent to an aqueous system is one of the oldest, most powerful and most popular of these cosolvent solubilization is particularly important for parenteral dosage forms where it is desirable to incorporate the required dose as a true solution in the smallest volume of liquid as possible.

A qualitative and intuitive way to understand the mechanism of cosolvency is that most cosolvent has hydrogen bond donor and / or acceptor groups as well as small hydrocarbon regions. Their hydrophilic hydrogen bonding groups ensure water miscibility while their hydrophobic hydrocarbon regions interfere with water hydrogen bonding network, reducing the overall intermolecular attraction of water. By disrupting water self-association, cosolvent reduces water ability to squeeze out non polar hydrophobic, compounds thus, increasing solubility. A different perspective is that by simply making the polar water environment more non polar like the solute cosolvent facilitate solubilization.

In the study five cosolvent namely ethanol, propylene glycol, glycerin, PEG-400 and PEG-200 were used to solubilize chlorzoxazone (Table 4.3.1-4.3.5) and indomethacin (Table 4.3.11-4.3.15). They resulted in various degree of improvement of solubilization of both drugs (Fig. 4.3.8 and 4.3.20).

As shown by the data the solubilization effect was dependent on both the concentration and the type of cosolvent used. An increase in concentration of cosolvent produces an exponential increase in drug solubility. Thus, solubilization effect was much significant at high cosolvent concentrations. In case of chlorzoxazone, it was noted that the maximum Solubilizing effect was observed with PEG-400 whereas glycerin exhibited the minimum effect. Ethanol, propylene glycol and PEG-200 showed intermediate effect. The solubility enhancement factor was found to 642.715, 429.618,

226.880, 104.039 and 6.371 for PEG-400, PEG-200, Ethanol PG and glycerin as 100% cosolvent respectively at 25 $^{\circ}$ C.

The similar results were obtained for indomethacin, maximum solubilizing effect was observed with PEG-400, an intermediate effect with PEG-200, Ethanol, Propylene glycol & minimum effect with glycerin as cosolvent. The enhancement ratio obtained by the five solvents was 4647.718, 3010.868, 751.016, 289.024 and 35.122 For PEG-400, PEG-200, ethanol, propylene glycol and glycerin respectively at 25°C.

The solubilizing power of the cosolvents were calculated by the slope of zero intercept curve of semi-log plot, plotted between log of solubility enhancement ratio verses fraction of cosolvent (Fig. 4.3.25 and 4.3.27). This facilitates comparison among both drug as well as different cosolvent. The results were shown in table 4.3.29 and 4.3.32. The solubilizing power of the five cosolvents PEG-400, PEG-200, Ethanol, Propylene glycol and glycerin was found 2.9125, 2.6381, 2.6991, 1.9671, 0.8255 for chlorzoxazone and 3.5561, 3.1526, 2.7589, 2.086, 1.8092 for indomethacin at 25°C. Thus the five cosolvents can be arranged regarding their solubilization power for chlorzoxazone according to the following rank: PEG-400 > Ethanol > PEG-200 > Propylene glycol> glycerin and for indomethacin according to the following rank: PEG-400 > PEG-200 > Ethanol > Propylene glycol> glycerin. This order is an agreement with the ranking of cosolvent with respects to their reported dielectric constant or solubility parameter (Table 4.3.25 and 4.3.24)

Cosolvent was reported to decrease the dielectric constant of water²⁰, the effect increasing with cosolvent concentration. The solubility results obtained in the present study are in accordance with the dielectric constant concept, which states that when the polarity of a solvent is decreased. It becomes a more favorable medium for dissolution of non polar or relatively non polar drugs²¹.

Some physico-chemical parameters of the solvents and the drugs used in the present study were either determined or calculated and given in table 4.3.21-4.3.28. Dielectric constants of the solvents show that the polarity of the

solvents varies as water > glycerin > propylene glycol > ethanol > polyethylene glycol 200 > polyethylene glycol 400. The solubility of the drugs decreases with an increase in the polarity of solvents. Thus, polarity of the solvent is an important factor governing the solubility of the drugs. Hydrophobicity of the solvents, measured as octanol-water partition coefficients (log P) (Table 4.3.28), also showed that the solubility increases with the hydrophobicity of the solvent. However, polarity and hydrophobicity are not the only factors involved, the ability of the solvent to form hydrogen bonds with the hetero-atoms in the drug molecule is another important factor governing the solubility of drugs, the same is true for higher solubility in ethanol. As the alkyl chain length in alcohols increases, their ability to form hydrogen bonds with the drug molecules decreases. The greater solubility of drugs in ethanol than in propylene glycol suggests that the solubility is also governed by the intermolecular interactions between the solvent molecules, which are expected to be stronger in glycols than in alcohols. In the case of glycols, the increase in solubility in moving from propylene glycol to polyethylene glycol suggests that the hydrophobic interactions are more important in governing the solubility of the studied drugs in glycols. The exceptionally high solubility of drugs in polyethylene glycol 400 is probably because of extensive hydrophobic interactions with the solvent because polyethylene glycol 400 has a long nonpolar part compared with other solvents

Water as solvent has some unique properties: high level of hydrogen bonding a sizable dielectric constant (80 at 20°C) and large surface tension (71 dynes/cm). The structure of PEG-400 is HO CH₂(CH₂OCH₂)n CH₂OH, where n is approximately 7 to 8 . This peculiar structure makes PEG-400 miscible with water through hydrogen bonding. The hydrophobic hydrocarbons region helps to break the hydrogen bonding between water molecules thus, reducing overall intermolecular interactions. In other words, PEG-400 may assist to reduce the dipole moment of water and allow hydrophobic compound to fit in²².

The most fundamental model for solubilization of a solute in a solvent involves liberation of solute molecules, creation of a hole in the solvent, and accommodation of the solute molecules in the solvent cavity²³. Work must be done to overcome the intermolecular forces of attraction in dissolving a solute. Four types of interactions namely solute-solvent, ion-dipole, dipole-dipole, and hydrogen bonding-hydrophobic have been reported

To investigate the effect of temperature on the solubility of chlorzoxazone and indomethacin by these cosolvents, the solubility study was performed at two temperature levels i.e. 25°C and 37°C. Elevation of the temperature was accompanied by a minor but detectable increase in the solubility of chlorzoxazone and indomethacin. The solubility of chlorzoxazone as in case of solids in general increased with temperature (endothermic) due to lowered stability of crystal lattice²⁴.

According to the thermodynamics for a spontaneous solubilization of a solute in a solvent, the associated ΔG must decrease or ΔG has to be negative. The dissolution of a solute involves the breaking of solid-state bonds in the solute, which is normally an endothermic process. The incorporation of the liberated solute molecules in the solvent cage is normally an exothermic process. One has to consider such enthalpic and entropic contributions in understanding the mechanism of solubilization.

The thermodynamic parameters of chlorzoxazone and indomethacin solubility in these co-solvents were calculated and the results are shown in table 4.3.31 and 4.3.34. The free energy change (ΔG) associated with the solubility process may indicate the type of reaction occurring between the solutes and solvents. The ΔG was calculated by the equation:

$$\Delta G = -2.303 \text{ RT log k}$$
 ...(5)

Where, k is ratio of the molar solubilities of the drug in water and cosolvent solution respectively²⁵. The greater the negative value of Δ G the better would be the solubility. The free energy change (Δ G) associated with the solubility of chlorzoxazone was found to be -16.02, -15.02, -13.44, -11.51

and -4.56 For PEG-400, PEG-200, ethanol, propylene glycol and glycerin respectively at 25° C. The free energy change (△G) associated with the solubility of indomethacin was found to be -20.92, -19.85, -16.41, -14.01 and -8.82 For PEG-400, PEG-200, ethanol, propylene glycol and glycerin respectively at 25° C.The negative values of ΔG can be arranged in the following rank: PEG-400> PEG-200> ethanol > PG> glycerin for chlorzoxazone and indomethacin (Table 4.3.31 and 4.3.34). This finding is in accordance with the solubilizing power of the five co-solvents under investigation (Table 4.3.29 and 4.3.31). The free energy change value showed that the increase in co-solvent concentration provided a more thermodynamically suitable environment for the solubility of the drug (ΔG) decrease). When non polar molecules are dissolved in water, hydrophobic association may lead to a structuring of the aqueous environment and to a decrease in entropy²⁶. The changes in any system are spontaneous when the free energy of the system decreases. This possibility is determined by three factors the change of heat ΔH (bonding strength), temperature (T) and entropy change ΔS (disordering or bond breaking). At a constant temperature, the free energy will be determined by the changes in the heat content and the entropy change, the equilibrium considered being between the same standard states.

$$\Delta G = \Delta H - T \Delta S \qquad \dots (6)$$

 $\underline{\Delta} H$ was determined using the integrated form of the van't Hoff equation.

$$\Delta H = 2.303 \log (S_{mix}/S_W)_2 RT_2T_1/(S_{mix}/S_W)_1 T_2-T_1 \dots (7)$$

Where S_{mix} and S_W are the molar solubilities of the drugs in co-solvent solutions and water; respectively, R is the gas constant, T is the absolute temperature and 1 and 2 refer to 25° and 37°, respectively. Alternatively the value of ΔH was determined by the slope of van't Hoff plot plotted between log K vs. 1/T (Fig. 4.3.26 and 4.3.28). The different thermodynamic parameters are listed in table 4.3.31 and 4.3.34. It is evident from Eq. 6 that the free

energy change (ΔG), which accompanies dissolution, is dependent on the value and sign of the change in enthalpy (ΔH)

The breaking up of water clusters surrounding the non-polar requires heat ($\pm\Delta$ H). Moreover, the dissolution process is endothermic one when Δ H is positive. Therefore, an increase in temperature from 25° to 37° caused an increase in chlorzoxazone or indomethacin solubility. In addition, the solute molecules become randomly spread through the medium during the dissolution process. This causes a disordering and an increase in the entropy associated with the system. The more of the positive the entropy changed is the greater the randomness or disorder degree of the system and the environment is thermodynamically more favorable \pm 1° (\pm 2° increase).

Solubility in Mixed-Solvent Systems

Solubilization behavior of chlorzoxazone and indomethacin in various proportions of mixed cosolvent systems Viz. Ethanol/PEG-200, Ethanol/PEG-400, Ethanol/propylene glycol and PEG-200/PEG-400 was also determined. Again, an exponential increase is solubility of both the drugs was observed when the proportion of high solubilizing power cosolvent is increased. The solvent with higher drug solubility in the pure state is referred to as the stronger solvent and the other as the weaker solvent in each case. The data are provided in tables 4.3.7-4.3.10 for chlorzoxazone and 4.3.17-4.3.20 for indomethacin. Examination of figures (Indomethacin Fig. 4.3.9-4.3.12 and chlorzoxazone Fig. 4.3.21-4.3.24) showed that there were significant deviations from linearity in solubility curves of different mixed cosolvent system for both the drugs. These deviations may be attributed to changes in the solute - cosolvent- cosolvent mixture that do not occur in the mixtures of the solute and the cosolvent or the solute and water. Many solutes reach a maximum solubility in certain proportion of cosolvent-solvent mixtures²⁸. It has been demonstrated that some solute exhibit peak solubilities at specific dielectric constant. In fact, multiple peaks have also been demonstrated for some solute - cosolvent-solvent mixtures. Such as observations might be due to solvate formation or change in crystalline from of solute28. The

Correlation of increased solubility with dielectric constants of the solvent mixtures showed that, in most cases solubility increased with a decrease in the dielectric constant of the mixture only up to a certain concentration of the stronger solvent, beyond which the solubility decreased. This effect occurs because drugs have some degree of polar character as well and maximum solubilization is a function of the relative polarity of the solute and the solvent. Moreover, factors other than the polarity of the solute and solvent are also involved.

The logarithmic relationship between total drug solubility in a mixed-solvent system and the volume fraction of the stronger solvent can be described by equation 8:

$$\log S_{mix} = \log S_{WS} + \sigma V_{SS} \qquad ...(8)$$

Where S_{mix} and S_{WS} are the solubilities of drug in solvent mixture and pure solvent, respectively. V_{ss} is the volume fraction of the stronger solvent and or is the solubilization power of the stronger solvent. The or value was obtained from the linear log $S_{\rm mix}$ versus $V_{\rm ss}$ plots (Fig. 4.3.29-4.3.32) The solubilization parameters for various drugs are given in table 4.3.35. For a given solvent system, the solvent system, the solubilization power (o) gave a quantitative estimate of the ability of the stronger solvent to increase the solubility of a drug in a given solvent. Some of the previous reports^{4,8,27} have shown that the solubilization power can be correlated to the solvent polarity or octanol-water partition coefficient of solute. The results show that the greater the difference in the polarity of the two solvents in a given mixed-solvent, the greater the solubilization power is. However, in a given mixed solvent system, the solubilization power does not bear a simple relationship to the polarity of the drugs, determined by their partition coefficients. Solubilization power data again shows that the structural factors other than the polarity/hydrophobicity of drugs are also involved. Maximum drug solubility was observed in PEG400ethanol mixtures in the case of both drugs.

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SURFACTANT SOLUBILIZATION 4.4

Surfactants are amphiphilic molecules, consisting of both hydrophilic and hydrophobic regions. These substances are known to play a vital role in many processes of interest in both fundamental and applied sciences. One important property of surfactants is the formation of colloidal-sized clusters in solutions, known as micelles, which have particular significance in Pharmacy because of their ability to increase the solubility of sparingly soluble substances in water¹. The ambivalence of amphiphiles towards an aqueous environment is responsible for the phenomenon of self-association of individual surfactant molecules resulting in a variety of micellar aggregate structures².

The utilization of micelles as drug carriers presents some advantages when compared to other alternatives such as soluble polymers and liposomes. Numerous drug delivery and drug targeting systems have been studied in an attempt to minimize drug degradation and loss, to prevent harmful side effects, and to increase drug bioavailability³⁻⁸. Micellar systems can solubilize poorly soluble drugs and thus increase their bioavailability, stay in the body (blood) long enough to provide gradual accumulation in the required area, and their sizes permit them to accumulate in areas with leaky vasculature. Moreover, specific ligands can be attached to their outer surface in order to optimize the controlled release and specificity of pharmacological effect. Another advantage is that the micelles can be obtained in an easy and reproducible manner in large scale⁹.

Therefore, the utilization of aqueous micellar solutions for drug solubilization can be advantageous for drug delivery purposes, with the possibility of increasing water solubility of poorly soluble drugs, improving bioavailability, reducing toxicity and other side effects, enhancing permeability across the physiological barriers, and substantial change in drug distribution⁹.

4.4.1 Phase Solubility Study of Chlorzoxazone in Surfactant Solution

The equilibrium phase solubility experiment was performed by the method reported by Higuchi and Connors¹⁰. surfactant solutions of Tween-20, Tween-80 and gelucire 44/14 of known w/v concentration were prepared by dissolving required amount of the respective Surfactant in water.

An excess quantity of chlorzoxazone was added to screw capped 15 ml glass culture tubes containing 10 ml of surfactant solution. The culture tubes were shaken vigorously for 15 minutes on touch type vortex mixer (Jyoti Scientific Industries Gwalior-474 009, India) and then the solutions were allowed to equilibrate with mechanically shaking and intermittent vortexing for 72 hrs at 25±2 °C and 37±2 °C in a Rotary flask shaker and shaker water bath (Jyoti scientific Industries Gwalior-474 009, India). After completion of 72 hrs, each culture tube is centrifuged for 10 min at 2000 rpm. The supernatant of each culture tube was filtered through 0.45μ membrane syringe filter (Sonar Axiva, Axiva Sichem Pvt. Ltd. Delhi, India.), filtrate diluted suitably with distilled water and analyzed spectrophotometrically at 280 nm against respective surfactant system diluted accordingly as blank. The solubility of chlorzoxazone was determined in triplicate.

Solubility of chlorzoxazone in mg/ml was calculated in different surfactant solution of different concentration and shown in table 4.4.1-4.4.3 and graphically presented in figure 4.4.1-4.4.3. Solubility enhancement ratios are calculated and reported in the same tables.

4.4.2 Phase Solubility Study of Indomethacin in Surfactant Solution

The equilibrium phase solubility experiment of indomethacin was performed by the same method as used for chlorzoxazone. Surfactant solutions were prepared as above. An excess quantity of indomethacin was equilibrated with the surfactant solutions for 72 hrs at 25±2 °C and 37±2 °C then centrifuged and filtered through 0.45 μ membrane syringe filter, The filtrate diluted suitably with distilled water and analyzed spectrophotometrically at 319.5 nm against respective surfactant system diluted accordingly as blank. The solubility of Indomethacin was determined in triplicate.

Solubility of indomethacin in mg/ml was calculated in different Surfactant solution of different concentration and shown in table 4.4.4-4.4.6 and graphically presented in figure 4.4.4-4.4.6. Solubility enhancement ratios are also calculated and reported in the same table.

Table 4.4.1: Solubility data of chlorzoxazone in various concentration of Tween-80

S. No	Conc. of	Solubility	in mg/ml	Enhancement Factor	
	Tween-80 (w/v)	At 25°C	At 37°C	At 25°C	At 37°C
1	0%	0.318±0.013	0.347±0.012	1.000	1.000
2	0.5%	0.390±0.031	0.433±0.056	1.224	1.246
3	1.0%	0.531±0.039	0.553±0.057	1.668	1,593
4	1.5%	0.582±0.041	0.636±0.049	1.827	1.832
5	2.0%	0.698±0.044	0.709±0.085	2.193	2.042
6	2.5%	0.756±0.101	0.856±0.102	2.375	2.465
7	3.0%	0.834±0.089	0.917±0.131	2.621	2.642
8	3.5%	0.945±0.064	1.062±0.095	2.967	3.058
9	4.0%	1.113±0.120	1.169±0.152	3.496	3.368
10	4.5%	1.184±0.242	1.369±0.108	3.720	3.942
11	5.0%	1.285±0.190	1.451±0.124	4.035	4.177

Table 4.4.2: Solubility data of chlorzoxazone in various concentration of Tween-20

S. No	Conc. of Tween-20 (w/v)	Solubility	Enhancement Factor		
J. 140		At 25° C	At 37° C	At 25° C	At 37° C
1	0%	0.318±0.013	0.347±0.012	1.000	1.000
2	0.1%	0.329±0.020	0.361±0.025	1.034	1.040
3	0.2%	0.343±0.032	0.383±0.047	1.078	1.103
4	0.3%	0.386±0.047	0.416±0.040	1.213	1.198
5	0.4%	0.402±0.055	0.452±0.055	1.263	1.302
6	0.5%	0.449±0.043	0.500±0.052	1.409	1.439

Table 4.4.3: Solubility data of chlorzoxazone in various concentration of Gelucire 44/14

S. No.	Conc. of		Solubility in mg/ml		Enhancement Factor	
	Gelucire 44/14 (w/v)	At 25°	С	At 37° C	At 25° C	At 37° C
1	0%	0.318±0.	013	0.347±0.012	1.000	1.000
2	2%	0.586±0.	039	0.829±0.087	1.841	2.387
3	4%	0.808±0.	041	1.103±0.101	2.538	3.176
4	6%	1.329±0.	137	1.520±0.244	4.174	4.376
5	8%	1.459±0.	107	2.150±0.290	4.583	6.191
6	10%	1.751±0.	122	2.447±0.306	5.501	7.046
7	20%	3.749±0.	283	4.778±0.275	11.779	13.759

Fig. 4.4.1: Solubility plot of chlorzoxazone in various concentration of Tween-80

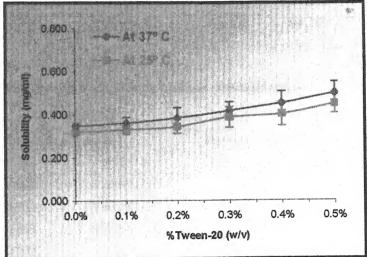


Fig. 4.4.2: Solubility plot of chlorzoxazone in various concentration of Tween-20

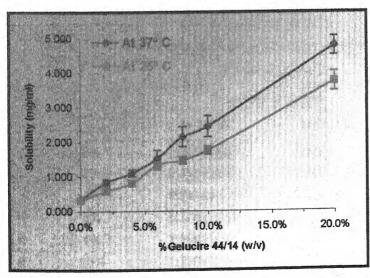


Fig. 4.4.3: Solubility plot of chlorzoxazone in various concentration of Gelucire 44/14

Table 4.4.4: Solubility data of indomethacin in various concentration of Tween-80

S. No.	Conc. of	Solubility	in mg/ml	Enhancem	ent Factor
3. 140.	Tween-80 (w/v)	At 25°C	At 37°C	At 25°C	At 37°C
1	0%	0.025±0.005	0.028±0.007	1.000	1.000
2	0.5%	0.246±0.043	0.316±0.118	9.851	11.130
3	1.0%	0.332±0.040	0.465±0.105	13.295	16.377
4	1.5%	0.434±0.038	0.702±0.128	17.397	24.741
5	2.0%	0.567±0.045	0.948±0.043	22.722	33.387
6	2.5%	0.797±0.071	1.027±0.052	31.911	36.166
7	3.0%	0.926±0.126	1.191±0.111	37.089	41.959
8	3.5%	0.994±0.096	1.410±0.239	39.809	49.654
9	4.0%	1.162±0.092	1.605±0.219	46.532	56.528
10	4.5%	1.234±0.111	1.738±0.123	49.415	61.213
11	5.0%	1.326±0.109	1.825±0.165	53.113	64.267

Table 4.4.5: Solubility data of indomethacin in various concentration of Tween-20

C No	Conc. of	Solubility	in mg/ml	Enhancem	ent Factor
S. No	Tween-20 (w/v)	At 25° C	At 37° C	At 25° C	At 37° C
1	0%	0.025±0.005	0.028±0.007	1.000	1.000
2	0.1%	0.409±0.052	0.455±0.046	16.378	16.025
3	0.2%	0.776±0.094	0.906±0.079	31.092	31.909
4	0.3%	1.074±0.114	1.186±0.101	42.987	41.786
5	0.4%	1.254±0.087	1.485±0.089	50.216	52.302
6	0.5%	1.439±0.109	1.713±0.128	57.635	60.364

Table 4.4.6: Solubility data of indomethacin in various concentration of Gelucire 44/14

**************************************	Conc. of	Solubility	in mg/ml	Enhancem	ent Factor
S. No.	Gelucire 44/14 — (w/v)	At 25° C	At 37° C	At 25° C	At 37° C
1	0%	0.025±0.005	0.028±0.007	1.000	1.000
2	2%	0.255±0.047	0.286±0.030	10.211	10.073
3	4%	0.541±0.040	0.615±0.045	21.664	21.660
4	6%	0.776±0.052	0.924±0.077	31.092	32.543
5	8%	1.005±0.057	1.201±0.118	40.235	42.299
6	10%	1.239±0.128	1.421±0.096	49.615	50.048
7	20%	2.377±0.185	2.873±0.037	95.189	101.184

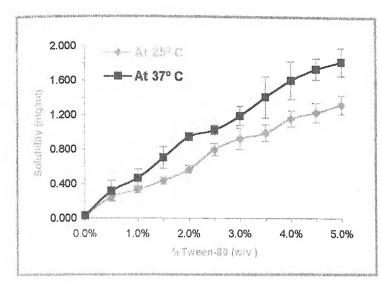


Fig. 4.4.4: Solubility plot of indomethacin in various concentration of Tween-80

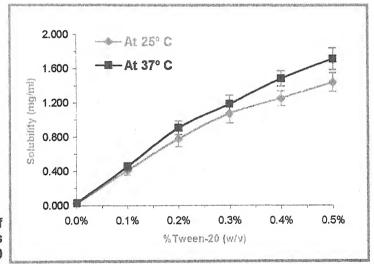


Fig. 4.4.5: Solubility plot of indomethacin in various concentration of Tween-20

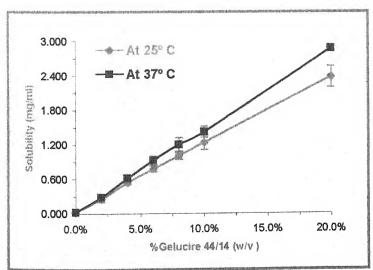


Fig. 4.4.6: Solubility plot of indomethacin in various concentration of Gelucire 44/14

4.4.3 Determination of Solution Properties

pН

The pH of saturated drug solution in different surfactant systems of various concentration was measured at 25±2°C using pH meter. The results are reported in table 4.4.7-4.4.9.

Viscosity

The viscosity of different surfactant systems of various concentration was determined using water as reference at 25±2°C by Ostwald Viscometer. The viscosity was calculated using following equation 11.

$$\eta_1 = \frac{\rho_1 t_1}{\rho_2 t_2} \times \eta_2$$

Where, η_1 and η_2 are viscosities, ρ_1 and ρ_2 are densities and t_1 and t_2 are times required for the flow of unknown cosolvent solution and reference liquid, respectively. The values are recorded in table 4.4.7-4.4.9.

Surface Tension

The surface tension of different surfactant systems of various concentration was determined using water as references at 25±2°C using Stalagmometer. The surface tension was calculated using following equation¹¹.

$$\gamma_1 = \frac{\rho_1 n_1}{\rho_2 n_2} \times \gamma_2$$

Where, γ_1 and γ_2 are surface tension, ρ_1 and ρ_2 are densities and n_1 and n_2 are number of drops formed of unknown hydrotrope solution and reference liquid water respectively. The values are recorded in table 4.4.7-4.4.9.

4.4.4 Calculation of Solubility Descriptors

Usually, the solubilization of a molecule by a surfactant can be evaluated based on two descriptors: molar solubilization capacity, χ , and micelle-water partition coefficient¹², K.

S. No.	%Tween-80 (w/v)	Surface Tension	Viscosity (Cps)		рН
	_	(Dynes/cm)	(-	Clz	Indo
1.	0	71.61	1.00	6.82	6.73
2.	0.5	29.26	1.03	6.78	6.51
3.	1.0	28.98	1.08	6.74	6.39
4.	1.5	28.79	1.09	6.72	6.32
5.	2.0	28.54	1.11	6.70	6.18
6.	2.5	28.29	1.11	6.67	6.02
7.	3.0	28.16	1.16	6.63	6.00
8.	3.5	27.91	1.16	6.58	5.91
9.	4.0	27.76	1.21	6.55	5.86
10.	4.5	27.52	1.26	6.51	5.61
11.	5.0	27.18	1.26	6.44	5.28

Table 4.4.8: Properties of tween-20 solution

S. No.	%Tween-20 (w/v)	Surface Tension	Viscosity (Cps)		рН
		(Dynes/cm)	(0,00)	Clz	Indo
1.	0	71.61	1.00	6.82	6.73
2.	0.1	30.11	1.01	6.63	6.41
3.	0.2	29.86	1.04	6.42	6.26
4.	0.3	29.77	1.06	6.38	6.02
5.	0.4	29.71	1.07	6.33	5.81
6.	0.5	28.56	1.09	6.27	5.35

Table 4.4.9: Properties of gelucire 44/14 solution

S. No.	%Gelucire 44/14 (w/v)	Surface Tension	Viscosity (Cps)		рН
	(,	(Dynes/cm)	(000)	Clz	Indo
1.	0	71.61	1.00	6.82	6.73
2.	2	35.12	1.05	6.81	6.63
3.	4	34.86	1.18	6.79	6.59
4.	6	34.29	1.29	6.78	6.29
5.	8	34.13	1.32	6.75	6.18
6.	10	33.96	1.38	6.73	6.11
7.	20	33.53	1.98	6.71	5.86

The χ value is defined as the number of moles of the solute (drug) that can be solubilized by one mol of micellar surfactant, and characterizes the ability of the surfactant to solubilize the drug. It can be calculated based on the general equation for micellar solubilization:

$$\chi = \frac{(S_{tot} - S_w)}{(C_{surf} - CMC)} \qquad \dots (1)$$

Where S_{tot} is the total drug solubility, S_W is the water drug solubility, C_{surf} is the molar concentration of surfactant in solution, and CMC is the critical micelle concentration 13 . Since above the CMC the surfactant monomer concentration is approximately equal to the CMC, the term (C_{surf} – CMC) is approximately equal to the surfactant concentration in the micellar form and, therefore, χ is equal to the ratio of drug concentration in the micelles to the surfactant concentration in the micellar form.

On the other hand, the micelle-water partition coefficient is the ratio of drug concentration in the micelle to the drug concentration in water for a particular surfactant concentration, as follows:

$$K = \frac{(S_{tot} - S_{w})}{S_{w}} \qquad \dots (2)$$

Combining Equations (1) and (2), we can relate the two solubility descriptors. Accordingly, for a given surfactant concentration:

$$K = \frac{\chi(C_{surf} - CMC)}{S_W} \qquad ...(3)$$

As can be seen, K is related to the water solubility of the compound, in contrary to χ^{13} . In order to eliminate the dependence of K on the surfactant concentration, a molar micelle-water partition coefficient, K_M , can be defined as follows:

$$K_{M} = \frac{\chi(1 - CMC)}{S_{W}} \qquad \dots (4)$$

The values of χ and K_M for the three surfactants for chlorzoxazone and indomethacin were calculated from their respective solubility curves (S_{tot} vs. C_{surf}) and Equations (1) and (4).

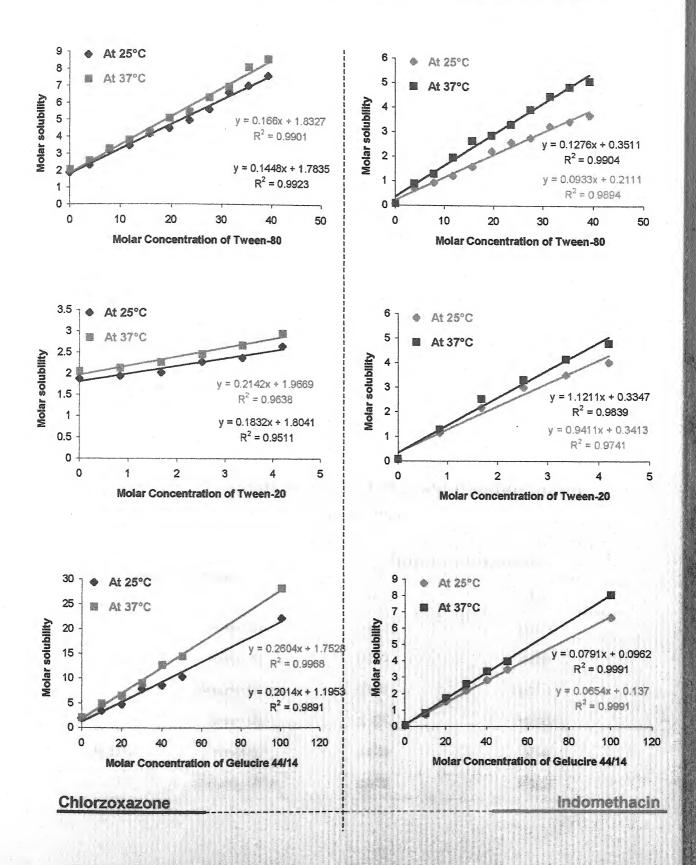


Fig. 4.4.7: Plot for determination of molar solubilization capacity (χ)

Table 4.4.10: Solubilization parameters of various surfactant systems for chlorzoxazone

Mice	ellar System	Solubilizat	ion parameters
	and Cyclonia	A	K _M
	Tween-20	0.183	97.419
At 25°C	Tween-80	0.145	77.221
	Gelucire 44/14	0.201	2884.4
	Tween-20	0.214	104.49
At 37°C	Tween-80	0.166	80.824
	Gelucire 44/14	0.26	3276.5

Table 4.4.11: Solubilization parameters of various surfactant systems for indomethacin

M	Han Creatans	Solubilizat	ion parameters
MICE	ellar System —	Α	K _M
	Tween-20	0.941	13478
At 25°C	Tween-80	0.093	1326
	Gelucire 44/14	0.065	937.1
	Tween-20	1.121	14129
At 37°C	Tween-80	0.128	1610
	Gelucire 44/14	0.079	996.8

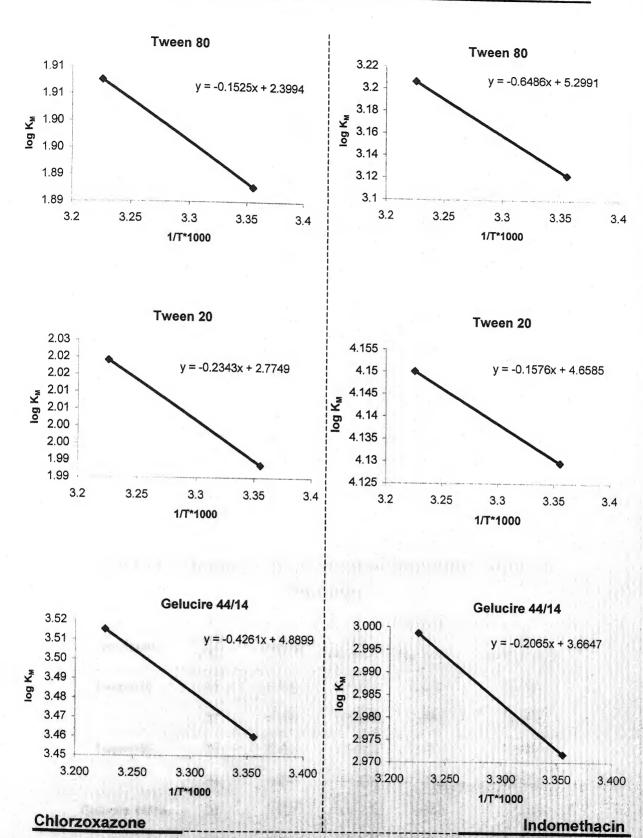


Fig. 4.4.8: Vant's Hoff plots of stability constants for drug-surfactant interactions

Table 4.4.12: Thermodynamic parameter for chlorzoxazone-surfactant interaction

Cosolvent	Temp. (°C)	Log K _M	ΔG (kJ.mol ⁻¹)	ΔH (kJ.mol ⁻¹)	∆\$ (J.mol ⁻¹ .K ⁻¹)
Tween-20	25	1.989	-11.345	4.486	53.125
	37	2.019	-11.518	4.486	51.628
Tween-80	25	1.888	-10.769	2.920	45.937
	37	1.908	-10.882	2.920	44.523
Gelucire 44/14	25	3.460	-19.739	8.159	93.617
	37	3.515	-20.056	8.159	91.012

Table 4.4.13: Thermodynamic parameter for indomethacin-surfactant interaction

Cosolvent	Temp. (°C)	Log K _M	ΔG (kJ.mol ⁻¹)	ΔH (kJ.mol ⁻¹)	ΔS (J.mol ⁻¹ .K ⁻¹)
Tween-20	25	4.130	-23.559	2,960	88.990
	37	4.150	-23.676	2.960	85.922
Tween-80	25	3.122	-17.813	12,419	101.450
	37	3.207	-18.294	12,419	99.074
Gelucire 44/14	25	2.972	-16.9536	3.954	70.160
	37	2.999	-17.1066	3.954	67.938

4.4.5 Results And Discussion

Solution in surfactant solution above critical micelle concentration offers one avenue for the formulation of poorly soluble drugs. Many drugs are also surface active at the air solution interface and can self-aggregate forming micelle or micelle like structure above critical concentration value 12,14. Indomethacin was also reported to have the similar activity¹⁵. An important property of micelles with particular significance in pharmacy is their ability to increase the solubility of poorly soluble drugs in water, thus increasing their bioavailability. In this work, the solubilization of chlorzoxazone and Indomethacin was studied in micellar solutions of three surfactants namely Tween-80, Tween-20 and Gelucire 44/14. Based on reported data on safe use in injectable formulation, Tween-80 was used up to the concentration of 5.0% v/v and Tween-20 was used up to 0.5% v/v. Gelucire 44/14 a well-defined mixture of mono-, di- and tri-glycerides and mono- and di-fatty acid esters of polyethylene glycol was used up to 20.0% w/v to find out its possible use in solubility enhancement of chlorzoxazone and Indomethacin. The results showed that, irrespective of the surfactant type, the solubility of chlorzoxazone and Indomethacin increased linearly with increasing surfactant concentration, as a consequence of the association between the drug and the micelles.

The amount of chlorzoxazone was solubilised to 0.449 ± 1.043 and $0.500\pm~0.052$ mg/ml by 0.5% tween-20 solution, 1.285 ± 0.190 and 1.451 ± 0.124 mg/ml by 5.0% tween-80 solution and 3.749 ± 0.283 and 4.778 ± 0.275 mg/ml by 20% w/v Gelucire 44/14 solution at 25° C and 37° C respectively. The enhancement ratio achieved by these three surfactants in the said concentration was found to be 1.409 and 1.439 for Tween-20, 4.035 and 4.177 for Tween-80 and 11.779 and 13.759 for gelucire 44/14 at 25° C and 37° C respectively. The values of molar solubilization capacity (χ) were obtained from the slope of solubility curves of chlorzoxazone with the three surfactants (S_{tot} vs. C_{surf}) (Fig. 4.4.7). The

highest value of molar solubilization capacity (χ) was obtained with Gelucire 44/14, χ = 0.201, followed by tween-20, χ = 0.183, and finally tween-80, χ = 0.145 at 25°C. Thus effectiveness of the three surfactant could be ranked as solubiliser for chlorzoxazone as Gelucire 44/14 >Tween-20 >Tween-80 (Table 4.4.10).

The result showed that Indomethacin was solubilised to 1.439 ± 0.109 and 1.713 ± 0.128 mg /ml by 0.5% tween-20 solution, 1.326 ± 0.109 and 1.825 ± 0.165 mg/ml by 5.0% tween-80 solution and 2.377 ± 0.185 and 2.873 ± 0.037 mg/ml by 20% w/v Gelucire 44/14 solution at 25° C and 37° C respectively. The enhancement ratio achieved by the three surfactants was found to be 57.635 and 60.365 for Tween-20, 53.113 and 64.267 for Tween-80 and 95.189 and 101.184 for Gelucire 44/14 in the above said concentration at 25° C and 37° C respectively. The values of molar solubilization capacity (χ) obtained from the slope of solubility curves (S_{tot} vs. C_{surf}) (Fig. 4.4.7). The highest value obtained with tween-20, χ = 0.941, intermediate with tween-80, χ = 0.093, and lowest with Gelucire 44/14, χ = 0.065 at 25° C. Thus effectiveness of the three surfactant could be ranked as solubilizer for Indomethacin as Tween-20>Tween-80>Gelucire 44/14 (Table 4.4.11).

Figure 4.4.9 present the results of chlorzoxazone and indomethacin solubility as a function of the surfactants Tween-20, Tween-80, and Gelucire-44/14 concentrations, respectively. Irrespective of the surfactant type, the solubility of both the drugs increased linearly with increasing surfactant concentration, as a consequence of the association between the drug and the micelles. It was considered that, the observed increase in drug solubility was only due to surfactant concentrations above the CMC, demonstrating that micellar solubilization was taking place. This relationship cannot be visualized from the solubilization curve of the drug in the surfactant systems because of the very low CMC of the nonionic surfactants.

The concentration of a monomeric amphiphile (surfactant molecule) at which micelles appear is called the Critical Micelle Concentration (CMC). The

occurrence of a CMC results from a delicate balance of intermolecular forces. The main attractive force results from the hydrophobic interaction among the nonpolar surfactant tails, whereas the main opposing repulsive force results from steric between the surfactant polar head groups ¹⁶. Micelles are known to have an anisotropic water distribution within their structure. In other words, the water concentration decreases from the surface towards the core of the micelle, with a completely hydrophobic (water excluded) core. These aggregates exhibit an interfacial region separating the polar bulk aqueous phase from the hydrocarbon-like interior. As a consequence, micellar solutions consist of a special medium in which hydrophobic, amphiphilic or ionic compounds may be solubilized and reagents may be concentrated or separated in aqueous solution ¹⁷. Moreover, the spatial position of a solubilized drug in a micelle will depend on its polarity: nonpolar molecules will be solubilized in the micellar core and substances with intermediate polarity will be distributed along the surfactant molecules in certain intermediate positions.

The low CMC of nonionic surfactants, combined with the low toxicity, makes this class of surfactants particularly interesting for solubilization of drugs. This nonionic surfactant presents a polyethylene oxide (PEO) hydrophilic head group that interacts with water through hydrogen bonds. In the case of indomethacin, it presents a terminal carboxyl group that may interact by hydrogen bonds with the PEO head groups. These interactions could drive the drug to the outer portion of the micellar core, in a way that the carboxyl group sticks to the palisade layer interacting with the PEO head groups, while the hydrophobic portion of the molecule stays preferentially in contact with the hydrophobic core^{9,18}. Therefore, being located mainly in the outer portion of the micelle core.

With relative small polar head groups, micelles formed by PEO nonionic surfactants have an alternative locus for solubilization. Although there is space for hydrating water in the outer parts of these micelles, there is virtually none close to the hydrocarbon core due to the crowding of the

polyoxyethylene chains¹⁹. As a result, a region that is largely purely polyoxyethylene rather than polyoxyethylene-water is formed, and may work as a site of solubilization of semipolar compounds, such as indomethacin. In this particular case, hydrogen bonds might be taking place between indomethacin and the polyoxyethylene head groups.

The increased solubility of the drug in non-ionic micellar solutions is not only a consequence of micelle-drug interaction, but also of the fraction of surfactant in the micellar form. For the nonionic surfactants, the molar fraction of surfactant in the micellar form is higher, since the CMC is much lower. In order to make this statement clear, the molar solubilization capacities of the surfactants, as well as the partition coefficients, were calculated and are presented in table 4.4.10. The molar micelle-water partition coefficients, K_{M} tween-20 = 97.419, K_M tween-80 =77.221 and K_M Gelucire-44/14 = 2884.4 for chlorzoxazone. Accordingly, the tendency of chlorzoxazone to partition preferentially with the Gelucire-44/14 micelle is higher than the tendency to partition with the tween-20 or tween-80 micelle. The tween-80 presented the lowest values of χ and $\textit{K}_{\textit{M}^{.}}$ as discussed earlier. For indometacin the molar micelle-water partition coefficients, K_M tween-20 = 13478 K_M tween-80 =1326 and K_M Gelucire-44/14 = 937.1 (Table 4.4.11), the tendency of indomethacin to partition preferentially with the tween-20 micelle is higher than the tendency to partition with the tween-80 or Gelucire-44/14 micelle. The Gelucire-44/14 presented the lowest values of χ and $K_{\rm M}$.

It was observed that these surfactants were found to be very less effective solubilizer for solubilization of chlorzoxazone but fairly good solubilizer for indomethacin. This may be due to self-association of indomethacin molecules and co micellization with surfactants. The process of transfer of drug from water to surfactant solution cannot be envisaged entirely as a simple solution mechanism, but rather as an interaction between surfactant and co solute i.e. comicellisation. Indomethacin was also known to form micelle like aggregates or mixed micelles. In solution of polyoxyethylene

containing surfactants namely tween-80 and tween-20, the Indomethacin may be associated with the ethylene oxide portion of the surfactant. The aggregation number of surfactant varies with the temperature due to a change of monomer hydrophobicity as well as difference in configuration of polyoxyethylene chain at different temperature. These effects could influence the mode of packing of the monomer in the micelles, which could lead to form larger micelles of larger volumes thus accommodating more drugs. The increase of temperature from 25 to 37°C was therefore expected to be accompanied by an enhanced solubility because of increasing the inherent solubility of the drug in the aqueous phase in addition to the increased amount of drug solubilized within the micelles. The result of chlorzoxazone and indomethacin solubilization showed a significant increase in solubility of the drug on increasing the temperature from 25 to 37°C in all the three surfactants system.

Knowledge of the thermodynamic parameters controlling solubilization is helpful to a better understanding of the mechanisms involved in this process. From the thermodynamic point of view, the solubilization can be considered as a normal partitioning of the drug between two phases, micelle and aqueous, and the standard free energy of solubilization, ΔG (J mol⁻¹), can be represented by the following expression⁹:

$$\Delta G = -2.303RT \log K_M \qquad ...(5)$$

Where R is the universal constant of the gases, T is the absolute temperature, and K_M is the molar partition coefficient between the micelle and the aqueous phase. The ΔG value was calculated for all systems, and the results are presented in table 4.4.12 and 4.4.13 for chlorzoxazone and indomethacin respectively for all systems studied.

 ΔG was negative, indicating spontaneous solubilization.

The enthalpy change ΔH was estimated from the slope of van't Hoff plot (plot of log K_M against 1/T, Fig. 4.4.8) and the entropy change ΔS is related to ΔG and ΔH as:

$$\Delta S = \frac{\Delta H - \Delta G}{T} \qquad(6)$$

The greater the value of ΔS the better would be the solubility. The negative value of ΔG can be arranged in following rank Gelucire 44/14 > Tween-20 > Tween-80 for chlorzoxazone (Table 4.4.12) and Tween-20 > Tween-80 > Gelucire 44/14 for indomethacin (Table 4.4.13). These findings were in accordance with molar solubilizing capacity and molar micelle-water partition coefficient of the three surfactants under investigation. Positive ΔH values show the dissolution process is endothermic. The breaking up of water clusters surrounding the non-polar portion requires heat (+ ΔH), therefore an increase in temperature from 25°C to 37°C caused an increase in drug solubility.

The values of ΔS were also positive indicating greater the randomness and disordering of the system or the solute molecules become more randomly spread through the dissolution medium during solubilization process. The more the positive entropy change is the greater the randomness or disordering degree of the system and the environment is thermodynamically more favorable.

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Development of Aqueous Injection Formulation

- 5.1 Formulation and Evaluation
- 5.2 Stability Studies
- 5.3 In vitro Studies
- 5.4 In vivo Studies

5.1 FORMULATION AND EVALUATION

Two key aspects of any successful injectable formulation are (i) to achieve the required drug concentration and (ii) drug should be chemically and physically stable in order to have sufficient shelf life. The ideal injectable formulation from in-vivo tolerability point of view is isotonic with physiological fluids and has a neutral pH 7.4 and thus the formulation scientist must use a wide variety of solubilization techniques. If the stability is insufficient to provide a two-year self-life, then the formulation scientist must change the solution condition to achieve both the solubility and stability.

The intravenous route is the most rapid and most bio-available method of getting a drug into systemic circulation. One of the greatest concerns with the use of solubilized systems is the systemic and local toxicity associated with their administration.

This part of the thesis focuses on formulation of stable aqueous injection of selected drugs to fulfill both the key aspects pointed above. The target strength for chlorzoxazone injection was 500 mg and for indomethacin injection was 50 mg, though in injectable dosage form dose may be reduced. It was evident from the result of preceding chapter on solubilization that the use of hydrotrope alone was not sufficient to make the injection of both drugs of required strength in specified volume (3 or 5 ml). The complexation and micellization also failed to achieve the desired concentration of both the drugs. Although the higher concentration of cosolvents were able to do so, but higher concentrations of cosolvents may not be justifiable because of toxicity aspect of relatively simpler indications as inflammation or muscular spasm. Most of the marketed formulation do employ high cosolvent concentration in indications like cancer were the quality of life of patient is already worst, hence attempt was made to solubilize the chlorzoxazone/indomethacin utilizing combination of hydrotrope and cosolvent or cosolvent and surfactant to achieve the desire dose in specified volume.

5.1.1 Selection of Solubilizer

The initial screening of hydrotrope was done on the basis of maximum solubility of drug in them when used alone and the visual changes that were observed during solubilizaton for the selection of cosolvent screening was done on the basis of precipitation formation immediately or when stored for 24 hrs in a refrigerator and 7 days at room temperature. Drug solution in cosolvents were diluted with distilled water in 1:10 and 1:100 dilution ratio and observed visually for precipitate formation and by observing the decrease in transmission at 650 nm spectrophotometrically. The selection of surfactant was based on maximum solubility of drug in them and also compatibility with cosolvents.

5.1.2 Formulation of Aqueous Injection of Chlorzoxazone

Three injectable formulation of chlorzoxazone namely C-1, C-2 and C-3 were prepared according to table 5.1.1. In all the formulations, 0.1% w/v sodium metabisulfite was added as an antioxidant. Other additives like chelating agent and buffering agent were not included in these formulations as they might lead to change in the solubility behavior and upset the basic solubility enhancement ratio. For the preparation of aqueous injection of chlorzoxazone, required amount of cosolvent mix (PEG-400 and ethanol) was taken in 100 ml borosil glass beakers and placed on a magnetic stirrer. In formulation C-3 benzyl alcohol was added to this stage, then weighed amount of chlorzoxazone was added into the beaker and stirred until complete dissolution. Sodium metabisulphite and buffer was dissolved separately in minimum amount of water and added to the drug solution. pH of the solution was adjusted to 7.8±0.1 with either benzoic acid or hydrochloric acid and sodium hydroxide solution then volume is made up to 50 ml with distilled water. The contents of these beakers were stirred for additional 1 hr on the magnetic stirrer for complete solubilization and equilibration then flushed with nitrogen for 15 minutes. These solutions were filtered through 0.45µ membrane filter (Sonar Axiva, Axiva Sichem Pvt. Ltd. Delhi, India.). The solutions were analyzed spectrophotometrically at 319.5 nm for drug content after appropriate dilutions with distilled water using the same vehicle as blank after appropriate dilution.

Table 5.1.1: Formulation of chlorzoxazone

	Sodium	0.1%	0.1%	0.1%
	phosphate buffer pH-7.8 (0.2 M)	1	10%	1
rzoxazone	Sodium benzoate	2%	· · · · · · · · · · · · · · · · · · ·	2%
ion of chlo	Benzyl alcohol	t «	ı	4%
Formulati	Ethanol	10%	10%	10%
Table 5.1.1: Formulation of chlorzoxazone	PEG-400	%02	%02	%08
	Chlorzoxazone	100mg/ml	100mg/ml	400mg/3ml
	Formulation	C-1	C-2	C-3
N 10 1-7 (10.01)	S O	.	ri '	3.

5.1.3 Formulation of Aqueous Injection of Indomethacin

Seven injectable formulation of indomethacin namely I-1a, I-1b, I-2a, I-2b, I-3a, I-3b and I-4 were prepared according to table 5.1.2. In all the formulations, 0.1% w/v sodium metabisulfite was added as an antioxidant. Other additives like chelating agent and buffering agent were not included in these formulations as they might lead to change in the solubility behavior and upset the basic solubility enhancement ratio. For the preparation of aqueous injection of indomethacin, required amount of cosolvent mix was taken in 100 ml borosil glass beakers and placed on a magnetic stirrer. Benzyl alcohol if the part of formulation was added to this stage, then weighed amount of indomethacin was added into the beaker and stirred until complete dissolution. Sodium metabisulphite and buffer was dissolved separetely in minimum amount of water and added to the drug solution. pH of the solution was adjusted to 6.9±0.2 with either benzoic acid or hydrochloric acid and sodium hvdroxide solution then volume is made to up 50 ml with distilled water. The contents of these beakers were stirred for additional 1 hr on the magnetic stirrer for complete solubilization and equilibration then flushed with nitrogen for 15 minutes. These solutions were filtered through 0.45 µm membrane filter (Sonar Axiva, Axiva Sichem Pvt. Ltd. Delhi, India.). The solutions were analyzed spectrophotometrically at 319.5 nm for drug content after appropriate dilutions with distilled water using the same vehicle as blank after appropriate dilution.

Indomethacin also has a great therapeutic value for closing hemodynamically significant patent ductus arteriosus (PDA) in premature infants¹ and to reduce the occurrence of intracranial or intraventricular hemorrhage in very-low-birth-weight neonates²⁻⁴. Thus additional three formulations (Table 5.1.3) of aqueous injection of indomethacin IPHB, ISB and INMD were also prepared, which contained 1mg/ml of indomethacin in 1M sodium p-hydroxy benzoate, 1.2 M sodium benzoate and 1.2 M nicotinamide solution respectively⁵⁻⁷. In all the formulations, 0.1% w/v sodium metabisulfite was added as an antioxidant.

Table 5.1.2: Formulation of indomethacin

No.	Formulation	S. No. Formulation Indomethacin		PEG-400 PEG-200	Propylene Glycol	Benzyl alcohol	Sodium benzoate	Tween-80	Sodium metabisulfite
_	<u>-</u> 1a	50mg/2ml	40%	ı	5	4%	2%	1	0.1%
7	L-1b	50mg/2ml	20%	ı		4%	2%	ı	0.1%
က်	I-2a	50mg/3ml	1		20%	4%	2%	1	0.1%
4	I-2b	50mg/3ml	1	1	%09	4%	2%	ı	0.1%
5.	I-3a	50mg/3ml	ı	40%	; 1	4%	2%	ı	0.1%
9	l-3b	50mg/3ml	1	20%		4%	2%		0.1%
7.	4	50mg/3ml	20%	1	1	ı	2%	2%	0.1%

Table 5.1.3: Additional formulation of indomethacin with target strength 1mg/ml using hydrotrope alone

S. No.	Formulation	Indomethacin	Sodium benzoate	Sodium p-hydroxy benzoate	Nicotinamide	Sodium metabisulfite
	ISB	1mg/ml	1.2 M	1	1	0.1%
2	IPHB	1mg/ml	ı	1.0 M	ı	0.1%
Ċ	INMD	1mg/ml	1	ı,	1.2 M	0.1%

Other additives like chelating agent and buffering agent were not included in these formulations as they might lead to change in the solubility behavior and upset the basic solubility enhancement ratio. For the preparation of aqueous injection of indomethacin, about 40 ml of distilled water was taken in 100 ml borosil glass beakers and placed on a magnetic stirrer. Then weighed amount of hydrotrope; sodium metabisulfite (0.1% w/v) and indomethacin were added into the beaker one by one after ensuring the complete dissolution of the former. pH of the solution was adjusted to 6.9±0.2 with either p- hydroxy benzoic acid, benzoic acid, hydrochloric acid or sodium hydroxide solution then volume is made up to 50 ml with distilled water. The contents of these beakers were stirred for additional 1 hr on the magnetic stirrer for complete solubilization and equilibration then flushed with nitrogen for 15 minutes. These solutions were filtered through 0.45 membrane filter (Sonar Axiva, Axiva Sichem Pvt. Ltd. Delhi, India.). The solutions were analyzed spectrophotometrically at 319.5 nm for drug content after appropriate dilutions with distilled water using the same vehicle as blank after appropriate dilution.

5.1.4 Treatment of Packing Material

Clear glass vials of 2 ml capacity were washed several times with deionized water jet then finally rinsed with distilled water. All these vials were placed in a perforated stainless steel box in inverted position and sterilized by dry heat in an oven at 180°C for 2 hrs. Rubber stoppers used for plugging the vials were first shacked in 0.2% liquid detergent solution for two hrs. Then washed several times with de-ionized water to remove any detergent residue of the detergent and finally rinsed with distilled water. These stoppers are then immersed in double strength hydrotrope and sodium metabisulfite solution and sterilized by autoclaving at 15 lbs pressure (121°C temperature) for 20 minutes. Finally, the stoppers are rinsed with freshly prepared sterile distilled water and dried in vacuum oven under aseptic condition.

5.1.5 Preparation of Aseptic Area

The walls and floor of aseptic room were thoroughly washed with detergent solution and water and then disinfected by mopping with 2.5% dettol and savion solution alternatively. The laminar airflow bench was cleaned with the same disinfectant and finally 70% v/v isopropyl alcohol was sprayed on to the surfaces as well as into the atmosphere. The aseptic room was then fumigated using a mixture of formaldehyde (40%) and pot. permagnate and the UV lights was switched on for overnight prior to filling of injections into vials.

5.1.6 Aseptic filtration

The aqueous solutions of indomethacin were prepared as above and sterilized by filtration through 0.2 μ membrane syringe filter (Axiva, Delhi, India.) previously sterilized by autoclaving.

5.1.7 Flushing with Nitrogen Gas

The sterile vial were pre and post flushed with sterile nitrogen during filling with sterile aqueous solution of indomethacin, stoppered immediately and sealed with 13mm.aluminium seals.

5.1.8 Evaluation of Prepared Injection Formulation

The prepared formulations were evaluated for drug content (assay), particulate method and sterility testing.

5.1.8.1 Drug content

The prepared dosage forms were estimated spectrophotometrically at 280 nm for chlorzoxazone and 319.5 nm for indomethacin content after suitable dilution.

5.1.8.2 Particulate matter

Sealed vials of the prepared dosage forms were inspected visually against black and white background in diffused florescent light for the presence of any particulate matter.

5.1.8.3 Sterility testing

Sterility testing was performed using three vials of each formulation by direct inoculation method (IP). The content of the vials was directly inoculated to the culture tubes containing sterile fluid thioglycollate media under aseptic conditions. Then the tubes were tightly plugged with cotton and kept into incubator at 35°C. The tubes were observed after 24 hr and upto 7 days for any growth or turbidity.

5.1.9 Results and Discussion

Based on the results of solubility determination studies, the formulation of aqueous injection of chlorzoxazone namely C-1, C-2 and C-3 were prepared. These parenteral formulations may be useful wherein the oral administration of chlorzoxazone is contraindicated or to uncooperative patients. At the same time there will be possibility of reducing the drug dose as well as the adverse effect.

Though it was difficult to attain desired dose 500 mg in 3 ml of injection volume, so higher concentration of PEG-400 with 10% ethanol was taken as cosolvent mix and 500 mg of chlorzoxazone was achieved in 5 ml of injection volume. The combination of PEG-400 and ethanol are common cosolvents vehicle that are considered safe for use in preparation of parenteral solutions. Also the solubilization study suggested that combination of PEG-400 and ethanol is highly effective solvent for chlorzoxazone having highest solubilizing power. 5% sodium benzoate was added as hydrotropic agent, which also served as buffer. In C-2 formulation benzoate buffer was replaced with 0.02 M phosphate buffer (pH 7.8). In formulation C-3 a ratio of 80% PEG-400 and 4% benzyl alcohol could be able to dissolve a high concentration of chlorzoxazone that is 400 mg / 3 ml. Benzyl alcohol 4% was added as it has synergistic effect in solubilization along with benzoic acid/sodium benzoate and also reduce pain on injection due to high cosolvent concentration. In each formulation 0.1% sodium metabisulfite was used as antioxidant.

Indomethacin is a non-steroidal anti-inflammatory and anti-pyretic drug that has been used extensively for various inflammatory diseases8. However, its clinical usefulness as an orally administered drug is restricted severely by serious systemic side effects and gastric irritation, e.g. disturbance, ulceration and bleeding. Administration by parenteral route offers the advantage of delivering the drug directly, bypassing these side effects and increasing local drug concentrations^{8,9}. On the basis of solubilization studies seven formulation of indomethacin with target strength of 50 mg in 2 ml or 3 ml injection volume were developed utilizing cosolvent and hydrotrope both and/or surfactant.

These parenteral formulations may be useful in-patient with rheumatic disorder, peptic ulcer etc. wherein the oral administration of indomethacin is contraindicated, with the possibility of reducing the drug dose as well as the adverse effect.

Cosolvents PEG-400, PEG-200 and PG were selected on the basis of their solubilizing power and in their acceptable concentration/proportion for injectable formulation. Among hydrotropes only sodium benzoate was selected, though the drug showed highest solubility in sodium p-hydroxy benzoate, but it was not used because presently there is no injectable formulation utilizing this hydrotrope so its acceptability was not known. Nicotinamide was also showed promising solubility enhancement, but it was also not used because of its high cost. Benzyl alcohol (4%) was added to each formulation as it has synergistic solubilization effect and also reduces pain on injection due to its local anesthetic properties. In formulation I-4 instead of benzyl alcohol, tween-80 (5% w/v) was utilized. Other surfactants such as tween-20, was not used because it produces yellowish colour during solubilization studies and gelucire 44/14 was not used because alone it was not able to produce desirable drug concentration and in combination of cosolvent, it was insoluble and produces opalescence.

Additionally three preparation of indomethacin with 1mg/ml target strength were also developed utilizing hydrotrope alone (Table 5.1.3). In each formulation, 0.1% sodium methabisulfite was added as antioxidant.

All the developed formulations were assayed for drug content and found within ±1% limit of stated drug amount. The pH of formulations were also found to be within limit. When observed against black and white background under diffused fluorescent light with naked eye all the sealed vials were found to be free from particulate matter.

Result of sterility testing, performed on the three vials of each formulation by direct inoculation method, showed that they were sterile as there was no turbidity or growth was observed in each culture tube after 7 days of inoculation. Thus it was concluded that the formulations developed were satisfactory and proceeded for further studies.

5.2 STABILITY STUDIES

A well-designed stability-testing plan is essential and pertinent part of the quality assurance curriculum. Ability of a formulation to retain properties in specified limits throughout its shelf life is referred as stability. Stability of a pharmaceutical product may be defined as a capability of a particular formulation, in a specific container, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications¹⁰.

Stability of a formulated product on shelf becomes an important factor in successful development of dosage form. A study of stability of a pharmaceutical product is essential for three main reasons; safety of the patient, legal requirement concern with identity, strength, purity and quality of the drug and to prevent the economic repercussions of marketing an unsuitable product¹¹.

5.2.1 Stability Studies as per ICH Guidelines

A systemic approach has been adopted for the presentation and evaluation of the stability information for physical, chemical and biological quality characteristics, including particular properties of the dosage form (dissolution rate for oral solid dosage forms). The guidelines were provided by the European Agency for the Evaluation of Medical Products (Human Medicine Evaluation unit, CPMP/ICH/380/95). The nature of degradation relationship will determine whether the data should be transferred for linear regression analysis. Usually the relationship can be presented by a linear quadratic, or cubic function on an arithmetic or logarithmic scale¹².

5.2.1.1 Accelerated stability testing

The products were also subjected for temperature dependent accelerated studies. The deterioration of active ingredients in pharmaceutical dosage forms may take place by hydrolysis, oxidation, reduction, recemerization, ring cleavage, decarboxylation and photolysis 13-15, oxidationreduction 16,17, pyrolysis 18 and hydrolysis 19-23 have been described in literature at a greater length, as these are most frequently encountered pathways involved

in degradation of pharmaceuticals¹⁴. Predictions were based on Arrhenius explanation, which could be applied to enumerate the effect of temperature on degradation rate.

The method of accelerated stability studies of pharmaceutical product based on the principles of chemical kinetics was demonstrated by Garrett and Carper²⁴. According to this technique, the degradation rate constant (K) at various elevated temperatures are obtained by plotting some function for residual drug concentration against time. From the slope of the plot, the degradation rate at that particular temperature is obtained. The logarithms of the specific rate of decompositions at different temperatures are then plotted against the reciprocal of the absolute temperature and the resulting line is extrapolated to room temperature to find the rate of degradation at room temperature. The technique is valid only when the breakdown is a thermal phenomenon. If the reaction rate is determined by photochemical reaction or if the decomposition is due to freezing or contamination with micro-organisms, an elevated temperature study obviously becomes of little use in predicting the life of a product. The effect of temperature is given by an equation proposed by Arrehenius

$$k = Ae^{-Ea/RT}$$

Where, k is a specific rate, A is constant known as a frequency factor, Ea is energy of activation, R is gas constant and T is the absolute temperature.

5.2.1.2 Expiry date (Shelf life)

The date placed on the container label of a drug product designed the time period to which a batch of the product is expected to remain within the approved shelf life specification if stored under defined conditions and after which it must not be used. In the pharmaceutical field, the 10% degradation of the drug is represented a reasonable limit of degradation of active ingredients. So, the time required for 10% degradation of the active

ingredient in the desired dosage form constitutes the shelf life of the product.

The shelf life of the drug can be calculated from the degradation rate constant at 25°C using the formula:

$$t_{10\%} = \frac{0.104}{k_{25}}$$
 Where $t_{10\%}$ = shelf life of the formulation.

5.2.2 Physical Stability Studies

The sealed vials of the formulations were visually inspected every day for 45 days under fluorescent light against black and white backgrounds to see the changes occurring, if any, in physical appearance of aqueous injection like color, turbidity, precipitation etc.²⁵ and also change in pH. These studies were performed under following conditions:

Room temperature in dark (RTD)

This involved keeping of vials at room temperature in dark place.

Freezing temperature in dark (FTD)

The vials were kept at 4±2°C in dark place.

Temperature cycling with shaking (TCS)

This method was designed to simulate storage and temperature condition and to induce any anticipated precipitation and check it in much shorter time. The vials were kept alternately at 45±2°C and 4±2°C for 24 hrs each and shaken everyday for 10 min on a metabolic shaker. Two vials of formulation were taken, one of which was kept at 45±2°C and the other at 4±2°C for first day, followed by subsequent temperature cycling and shaking as described above.

All the vials of different formulations were subjected to the aforesaid physical stability-testing programme for 30 days. The observations are recorded in table 5.2.1 and 5.2.2 for chlorzoxazone and indomethacin respectively.

Table 5.2.1: Physical Stability Data for Formulation of Chlorzoxazone

		Colour		р	рН		Time in days after which first precipitate appeared	
S. No.	Formulation	Initial	After 45 days	initial	After 45 days	RTD	FTD	TCS
1.	C-1	CLS	CLS	7.81	7.79	No ppt.	No ppt.	31
2.	C-2	CLS	VSB	7.84	7.81	No ppt.	No ppt.	39
3.	C-3	CLS	CLS	7.85	7.83	No ppt.	No ppt.	No ppt.

CLS=colourless, VSB=very slight browncolour, ppt.= precipitate

Table 5.2.2: Physical Stability Data for Formulation of Indomethacin

S		Colour		рН		Time in days after which first precipitate appeared		
No. Formulat	Formulation	Initial	After 45 days	Initial	After 45 days	RTD	FTD	TCS
1.	I-1a	CSY	NACC	6.91	6.85	No ppt.	31	13
2.	l-1b	CSY	VSCC	6.95	6.87	No ppt.	39	19
3.	I-2a	CSY	NACC	6.92	6.88	No ppt.	No ppt.	43
4.	l-2b	CSY	NACC	6.96	6.83	No ppt.	No ppt.	No ppt.
5.	I-3a	CSY	NACC	7.08	6.92	No ppt.	No ppt.	No ppt.
6.	I-3b	CSY	NACC	7.04	6.89	No ppt.	No ppt.	No ppt.
7.	1-6	CSY	VSCC	7.11	6.95	No ppt.	No ppt.	No ppt.
8.	ISB	VSCY	NACC	6.95	6.92	No ppt.	No ppt.	41
9.	IPHB	VSCY	VSCC	6.89	6.85	No ppt.	31	14
10.	INMD	VSCY	NACC	7.02	7.00	No ppt.	No ppt.	42

CSY= characteristic slight yellow colour, VSCY= very slight characteristic yellow colour, NACC= no apparent colour change, VSCC= very slight colour change, ppt.= precipitate

5.2.3 Chemical Stability Studies

The developed formulations were subjected to exhaustive chemical stability at elevated temperatures 37±2°C, 45±2°C and 60±2°C in thermostatically controlled ovens for a period of 45 days²⁶. The formulations were analyzed spectrophotometrically initially and at intervals of 1, 3, 7, 14, 21, 30 and 45 days to calculate the drug content. The percentage drug remaining for each formulation at different time intervals as well as at different temperatures was calculated considering the initial drug content for each formulation to be 100%. For formulation of chlorzoxazone these values are shown in table 5.2.3-5.2.5 (For formulation C-1, C-2 and C-3 respectively) and plotted as natural log percentage drug remaining vs. time (days) (Fig. 5.2.1-5.2.3) to obtained the degradation rate constant K (Table 5.2.6) at different temperature. Arrhenius plots were constructed between log of degradation rate constant (K) vs. the reciprocal of the absolute temperature (1/T) (Fig. 5.2.4). From the Arrhenius plots, the K values at 25°C were determined by extrapolating the graph. The time period required for 10%, degradation of drug ($t_{10\%}$) for each formulation was calculated (Table 5.2.6).

For formulation of indomethacin values of percentage drug remaining at different time interval are shown in table 5.2.7-5.2.16 (For formulation I-1a, I-1b, I-2a, I-2b, I-3a, I-3b, I-4, ISB, IPHB and INMD respectively). The plots of natural log percentage drug remaining vs. time (days) (Fig. 5.2.5-5.2.14) were constructed to obtained the degradation rate constant K (Table 5.2.17) at different temperature. Arrhenius plots were constructed between log of degradation rate constant (K) vs. the reciprocal of the absolute temperature (1/T) (Fig. 5.2.15 and 5.2.16). From the Arrhenius plots, the K values at 25°C were determined by extrapolating the graph. The time period required for 10%, degradation of drug (t_{10%}) for each formulation was calculated (Table 5.2.17).

Table 5.2.3: Accelerated Chemical stability data of formulation C-1

S. No.	No. of Days	Percentage Chlorzoxazone remaining* in formulation C-1 s at temperature			
		37±2°C	45±2°C	60±2°C	
1	0	100.00 (4.6052)	100 (4.6052)	100.00 (4.6052)	
2	1	99.75 (4.6027)	99.68 (4.6020)	99.43 (4.5995)	
3	3	99.56 (4.6008)	99.36 (4.5987)	98.77 (4.5928)	
4	7	99.21 (4.5972)	99.01 (4.5952)	98.12 (4.5862)	
5	14	98.68 (4.5919)	98.12 (4.5862)	96.27 (4.5672)	
6	21	97.82 (4.5831)	97.27 (4.5775)	94.94 (4.5532)	
7	30	96.93 (4.5740)	95.64 (4.5606)	92.86 (4.5311)	
8	45	95.67 (4.5609)	94.19 (4.5453)	90.08 (4.5007)	

4.62 ▲ At 37°C × At 45°C × At 60°C 4.60 LN % drug remaining 4.58 y = -0.000976x + 4.604227 $R^2 = 0.997121$ 4.56 4.54 = -0.001334x + 4.604010 $R^2 = 0.993750$ 4.52 4.50 = -0.002290x + 4.601605 $R^2 = 0.996758$ 4.48 20 30 40 50 60 10 0 **Time in Days**

Fig. 5.2.1: Plot for determination of accelerated stability constant of formulation C-1

^{*}Values are mean of three observations

Table 5.2.4: Accelerated Chemical stability data of formulation C-2

S. No.	No. of Days	at temperature				
		37±2°C	45±2°C	60±2°C		
1	0	100.00 (4.6052)	100 (4.6052)	100.00 (4.6052)		
2	1	99.74 (4.6026)	99.45 (4.5997)	99.24 (4.5975)		
3	3	99.33 (4.5984)	99.18 (4.5969)	98.67 (4.5918)		
4	7	98.57 (4.5908)	98.67 (4.5918)	97.46 (4.5794)		
5	14	98.12 (4.5862)	97.82 (4.5831)	96.19 (4.5663)		
6	21	97.29 (4.5777)	97.14 (4.5762)	94.73 (4.5510)		
7	30	96.97 (4.5744)	95.86 (4.5629)	93.15 (4.5342)		
8	45	95.82 (4.5625)	93.57 (4.5387)	91.62 (4.5176)		

4.62 At 37°C × At 45°C × At 60°C 4.60 4.58 LN % drug remaining y = -0.000915x + 4.601048 $R^2 = 0.957404$ 4.56 y = -0.001381x + 4.602694 $R^2 = 0.992687$ 4.54 = -0.001935x + 4.597161 4.52 $R^2 = 0.969276$ 4.50 20 30 40 50 60 0 10 **Time in Days**

Fig. 5.2.2: Plot for determination of accelerated stability constant of formulation C-2

^{*}Values are mean of three observations

Table 5.2.5: Accelerated Chemical stability data of formulation C-3

S. No.	No. of Days	Percentage Chlorzoxazone remaining* in formulation C-3 sto			
	Days	37±2°C	45±2°C	60±2°C	
1	0	100	100.00	100.00	
	O	(4.6052)	(4.6052)	(4.6052)	
2 1	1	99.58	99.78	99.36	
	1	(4.6010)	(4.6030)	(4.5987)	
3 3	2	99.05	98.96	98.59	
	3	(4.5956)	(4.5947)	(4.5910)	
4 7	7	98.64	98.74	98.23	
4	1	(4.5915)	(4.5925)	(4.5873)	
5 14	97.91	98.47	96.87		
5	14	(4.5840)	(4.5898)	(4.5734)	
6	21	97.13	97.89	95.52	
0	21	(4.5761)	(4.5838)	(4.5593)	
7	30	96.23	97.24	93.61	
,	30	(4.5667)	(4.5772)	(4.5391)	
8	45	94.42	95.97	90.57	
0	40	(4.5478)	(4.5640)	(4.5061)	

*Values are mean of three observations

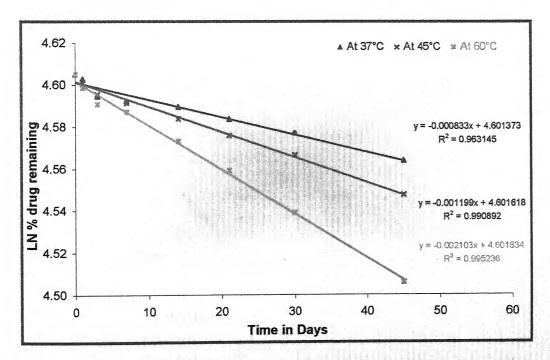
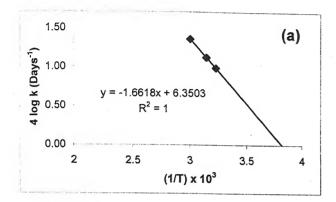
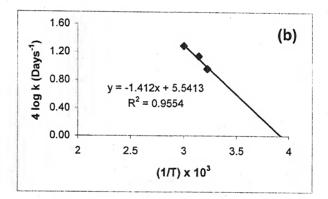


Fig. 5.2.3: Plot for determination of accelerated stability constant of formulation C-3

Table 5.2.6: Degradation rate constant and shelf life of formulated products

Formulations	Degradation at c	Shelf life (day At 25±2°C		
-	37 ±2°C	45 ±2°C	60 ±2°C	By Arrhenius plot
C-1	9.76	13.34	22.9	175.083
C-2	9.15	13.81	19.35	163.6774
C-3	8.33	11.99	21.03	211.273





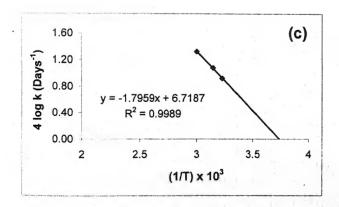


Fig. 5.2.4: Arrehenius plot for (a) C-1 (b) C-2 and (c) C-3 formulations

Table 5.2.7: Accelerated Chemical stability data of formulation I-1a

S. No.	No. of Days	Percentage Indomethacin remaining* in formulation I-1a s				
		37±2° C	45±2° C	60±2° C		
1	0	100.00 (4.6052)	100 (4.6052)	100 (4.6052)		
2	1	99.35 (4.5986)	99.38 (4.5990)	99.23 (4.5974)		
3	3	99.46 (4.5998)	99.1 (4.5961)	98.86 (4.5937)		
4	7	98.89 (4.5940)	98.71 (4.5922)	97.87 (4.5836)		
5	14	98.47 (4.5898)	97.84 (4.5833)	95.94 (4.5637)		
6	21	97.94 (4.5844)	95.76 (4.5618)	92.64 (4.5287)		
7	30	96.27 (4.5672)	94.81 (4.5519)	90.93 (4.5101)		
8	45	95.04 (4.5543)	92.26 (4.5246)	86.72 (4.4627)		

^{*}Values are mean of three observations

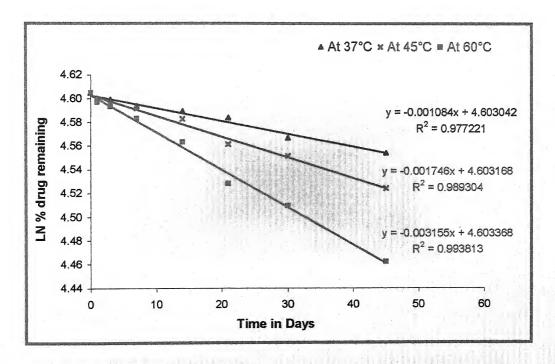


Fig. 5.2.5: Plot for determination of accelerated degradation rate constant of formulation I-1a

Table 5.2.8: Accelerated Chemical stability data of formulation I-1b

S. No.	No. of Days	Percentage Indomethacin remaining* in formulation I-1b stored at temperature							
		37±2°C	45±2°C	60±2°C					
1	0	100.00 (4.6052)	100.00 (4.6052)	100.00 (4.6052)					
2	1	99.46 (4.5998)	99.15 (4.5966)	99.24 (4.5975)					
3	3	99.16 (4.5967)	98.97 (4.5948)	98.56 (4.5907)					
4	7	98.79 (4.5930)	98.26 (4.5876)	97.13 (4.5761)					
5	14	98.41 (4.5891)	97.48 (4.5796)	95.58 (4.5600)					
6	21	97.67 (4.5816)	95.26 (4.5566)	92.37 (4.5258)					
7	30	96.92 (4.5739)	93.49 (4.5379)	89.54 (4.4947)					
8	45	94.16 (4.5450)	92.33 (4.5254)	84.17 (4.4328)					

*Values are mean of three observations

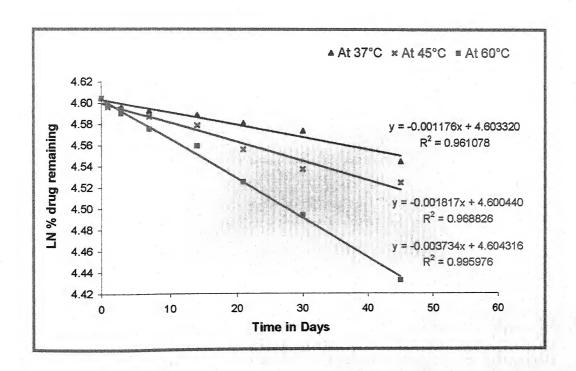


Fig. 5.2.6: Plot for determination of accelerated degradation rate constant of formulation I-1b

Table 5.2.9: Accelerated Chemical stability data of formulation I-2a

S. No.	No. of Days -	Percentage Indomethacin remaining* in formulation I-2a store at temperature						
	Days	37±2° C	45±2° C	60±2° C				
1	0	100.00	100.00	100.00				
•	Ü	(4.6052)	(4.6052)	(4.6052)				
2	1	99.54	99.53	99.08				
2	'	(4.6006)	(4.6005)	(4.5959)				
3	3 3	99.34	99.13	98.76				
3	9	(4.5985)	(4.5964)	(4.5927)				
4	7	98.97	98.61	96.99				
7	,	(4.5948)	(4.5912)	(4.5746)				
5	14	98.59	97.76	94.15				
5	17	(4.5910)	(4.5825)	(4.5449)				
6	21	97.83	95.88	91.26				
U	۷1	(4.5832)	(4.5631)	(4.5137)				
7	30	97.31	94.27	88.67				
1	30	(4.5779)	(4.5462)	(4.4849)				
8	45	96.09	93.15	82.19				
0	40	(4.5653)	(4.5342)	(4.4090)				

*Values are mean of three observations

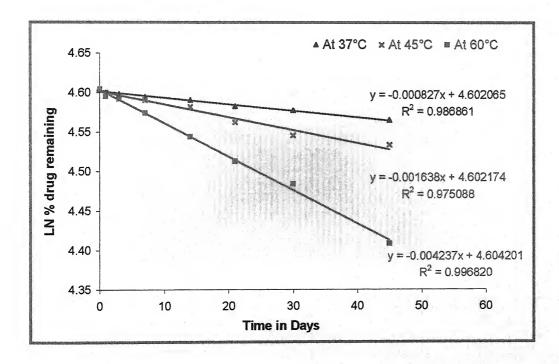


Fig. 5.2.7: Plot for determination of accelerated degradation rate constant of formulation I-2a

Table 5.2.10: Accelerated Chemical stability data of formulation I-2b

S. No.	No. of Days	Percentage Indomethacin remaining* in formulation I-2b store at temperature						
		37±2° C	45±2° C	60±2° C				
1	0	100.00 (4.6052)	100.00 (4.6052)	100.00 (4.6052)				
2	1	99.83 (4.6035)	99.35 (4.5986)	99.16 (4.5967)				
3	3	99.48 (4.6000)	99.46 (4.5998)	98.29 (4.5879)				
4	7	99.16 (4.5967)	98.89 (4.5940)	97.57 (4.5806)				
5	14	98.88 (4.5939)	98.47 (4.5898)	96.24 (4.5668)				
6	21	98.27 (4.5877)	97.94 (4.5844)	94.78 (4.5516)				
7	30	97.89 94.5838)	96.27 (4.5672)	92.66 (4.5289)				
8	45	97.01 (4.5748)	95.14 (4.5553)	90.14 (4.5014)				

^{*}Values are mean of three observations

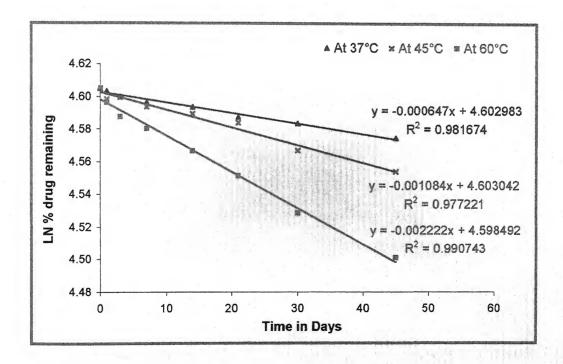


Fig. 5.2.8: Plot for determination of accelerated degradation rate constant of formulation I-2b

Table 5.2.11: Accelerated Chemical stability data of formulation I-3a

S. No.	No. of Days	Percentage Indomethacin remaining* in formulation I-3a stored at Temperature						
		37±2° C	45±2° C	60±2° C				
1	0	100.00 (4.6052)	100.00 (4.6052)	100 (4.6052)				
2	1	99.33 (4.5984)	99.64 (4.6016)	99.18 (4.5969)				
3	3	99.21 (4.5972)	98.96 (4.5947)	98.76 (4.5927)				
4	7	98.92 (4.5943)	98.26 (4.5876)	97.85 (4.5834)				
5	14	98.37 (4.5887)	97.31 (4.5779)	95.43 (4.5584)				
6	21	97.89 (4.5838)	95.49 (4.5590)	92.11 (4.5230)				
7	30	97.01 (4.5748)	93.99 (4.5432)	89.26 (4.4916)				
8	45	95.56 (4.5598)	92.28 (4.5248)	83.46 (4.4244)				

▲ At 37°C × At 45°C 4.65 4.60 LN % drug remaining y = -0.000913x + 4.601605 $R^2 = 0.984089$ 4.55 y = -0.001810x + 4.6016344.50 $R^2 = 0.986211$ y = -0.003952x + 4.6067164.45 $R^2 = 0.994958$ 4.40 10 20 30 40 50 60 0 **Time in Days**

Fig. 5.2.9: Plot for determination of accelerated degradation rate constant of formulation I-3a

^{*}Values are mean of three observations

Table 5.2.12: Accelerated Chemical stability data of formulation I-3b

S. No.	No. of Days –	Percentage Indomethacin remaining* in formulation I-3b stored at temperature						
	Duyo	37±2° C	45±2° C	60±2° C				
1	0	100.00 (4.6052)	100.00 (4.6052)	100.00 (4.6052)				
2	1	99.34 (4.5985)	99.53 (4.6005)	98.94 (4.5945)				
3	3	99.41 (4.5993)	99.13 (4.5964)	98.27 (4.5877)				
4	7	99.02 (4.5953)	98.36 (4.5886)	96.75 (4.5721)				
5	14	98.54 (4.5905)	97.72 (4.5821)	94.28 (4.5463)				
6	21	97.98 (4.5848)	95.94 (4.5637)	90.79 (4.5085)				
7	30	97.26 (4.5774)	94.34 (4.5469)	88.26 (4.4803)				
8	45	95.77 (4.5619)	92.51 (4.5273)	84.81 (4.4404)				

^{*}Values are mean of three observations

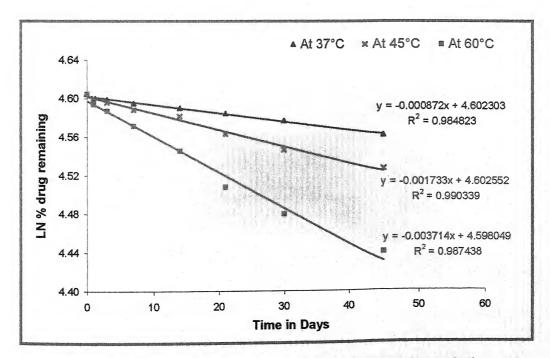


Fig. 5.2.10: Plot for determination of accelerated degradation rate constant of formulation I-3b

Table 5.2.13: Accelerated Chemical stability data of formulation I-4

S. No.	No. of Days	Percentage Indomethacin remaining* in formulation I-6 stored at temperature						
	Dayo	37±2° C	45±2° C	60±2° C				
1	0	100.00 (4.6052)	100 (4.6052)	100.00 (4.6052)				
2	1	99.47 (4.5999)	99.22 (4.5973)	99.04 (4.5955)				
3	3	99.41 (4.5993)	99.01 (4.5952)	98.12 (4.5862)				
4	7	98.97 (4.5948)	98.42 (4.5892)	97.35 (4.5783)				
5	14	98.53 (4.5904)	96.73 (4.5719)	95.76 (4.5618)				
6	21	98.02 (4.5852)	94.63 (4.5500)	92.34 (4.5255)				
7	30	97.57 (4.5806)	92.82 (4.5307)	89.17 (4.4905)				
8	45	95.94 (4.5637)	90.45 (4.5048)	86.46 (4.4597)				

^{*}Values are mean of three observations

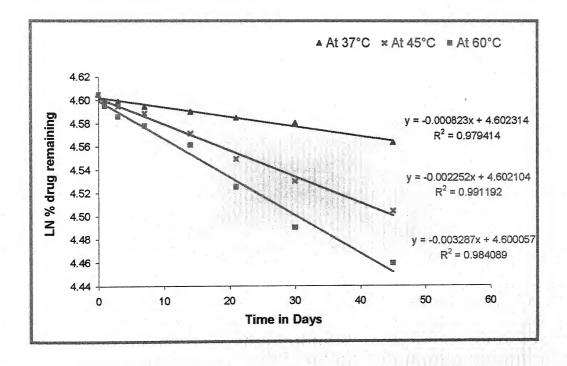


Fig. 5.2.11: Plot for determination of accelerated degradation rate constant of formulation I-4

Table 5.2.14: Accelerated Chemical stability data of formulation ISB

S. No.	No. of Days	Percentage Indomethacin remaining* in formulation ISB stored at temperature						
		37±2°C	45±2°C	60±2°C				
1	0	100.00 (4.6052)	100.00 (4.6052)	100.00 (4.6052)				
2	1	99.68 (4.6020)	99.27 (4.5978)	99.13 (4.5964)				
3	3	99.33 (4.5984)	99.14 (4.5965)	98.87 (4.5938)				
4	7	98.84 (4.5935)	98.42 (4.5892)	97.99 (4.5849)				
5	14	98.26 (4.5876)	97.67 (4.5816)	96.46 (4.5691)				
6	21	97.73 (4.5822)	96.82 (4.5729)	95.16 (4.5556)				
7	30	96.85 (4.5732)	95.88 (4.5631)	93.24 (4.5352)				
8	45	95.86 (4.5629)	95.86 94.23					

Values in parenthesis indicates natural log values *Values are mean of three observations

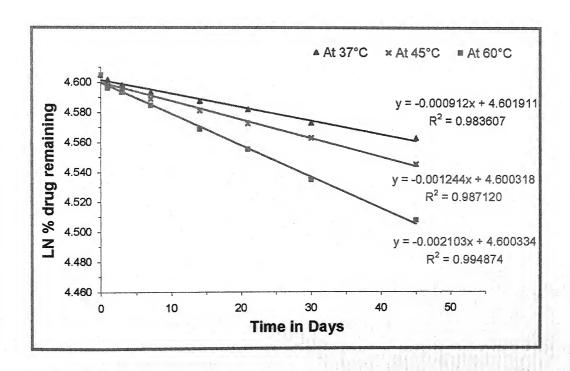


Fig. 5.2.12: Plot for determination of accelerated degradation rate constant of formulation ISB

S. No.	No. of Days	Percentage Indomethacin remaining* in formulation IPHB store at temperature						
	,-	37±2°C	45±2°C	60±2°C				
1 0		100 (4.6052)	100 (4.6052)	100 (4.6052)				
2	1	99.33 (4.5984)	99.25 (4.5976)	98.99 (4.5950)				
3	3	98.9 (4.5941)	98.88 (4.5939)	98.58 (4.5909)				
4	7	98.56 (4.5907)	98.26 (4.5876)	97.66 (4.5815)				
5	14	97.68 (4.5817)	96.87 (4.5734)	96.15 (4.5659)				
6	21	96.83 (4.5730)	95.79 (4.5622)	94.36 (4.5471)				
7	30	95.59 (4.5601)	94.42 (4.5478)	92.17 (4.5236)				
8	45	93.65 (4.5396)	91.83 (4.5199)	88.23 (4.4799)				

*Values are mean of three observations

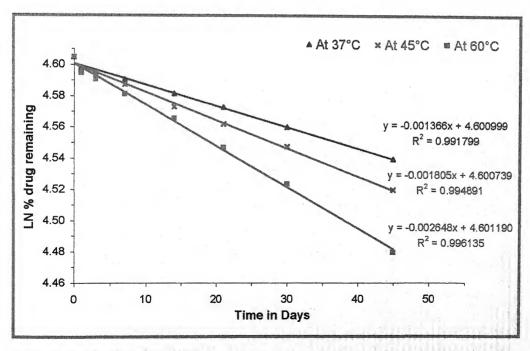


Fig. 5.2.13: Plot for determination of accelerated degradation rate constant of formulation IPHB

Table 5.2.16: Accelerated Chemical stability data of formulation INMD

S. No.	No. of Days	Percentage Indomethacin remaining* in formulation INMD store at temperature						
	Duyo	37±2°C	45±2°C	60±2°C				
1	0	100 (4.6052)	100 (4.6052)	100 (4.6052)				
2	1	99.33 (4.5984)	99.22 (4.5973)	99.19 (4.5970)				
3	3	99.05 98.97 (4.5956) (4.5948		98.87 (4.5938)				
4	7	98.83 (4.5934)	98.56 (4.5907)	98.03 (4.5853)				
5	14	98.42 (4.5892)	97.86 (4.5835)	96.88 (4.5735)				
6	21	97.75 (4.5824)	97.14 (4.5762)	95.66 (4.5608)				
7	30	97.03 (4.5750)	96.21 (4.5665)	93.94 (4.5427)				
8	45	96.34 (4.5679)	94.87 (4.5525)	91.54 (4.5168)				

*Values are mean of three observations

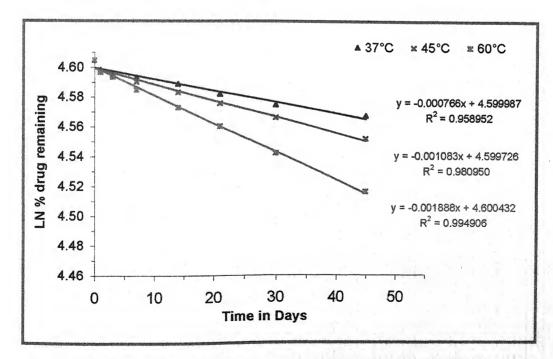


Fig. 5.2.14: Plot for determination of accelerated degradation rate constant of formulation INMD

Table 5.2.17: Degradation rate constant and shelf life of Indomethacin formulated products

Formulations	Degradation at d	Shelf life (day) at 25 ±2° C		
	37 ±2°C	37 ±2°C 45 ±2°C		by Arrhenius plot
l-1a	10.84	17.46	31.55	171.0
I-1b	11.76	18.17	37.34	172.2
I-2a	8.27	16.38	42.37	311.6
I-2b	6.47	10.84	22.22	318.8
I-3a	9.13	18.1	39.52	247.4
1-3b	8.72	17.33	37.14	255.7
1-4	8.23	22.52	32.87	218.6
ISB	9.12	12.44	21.03	185.1
IPHB	13.66	18.05	26.48	109.8
INMD	7.66	10.83	18.88	227.7

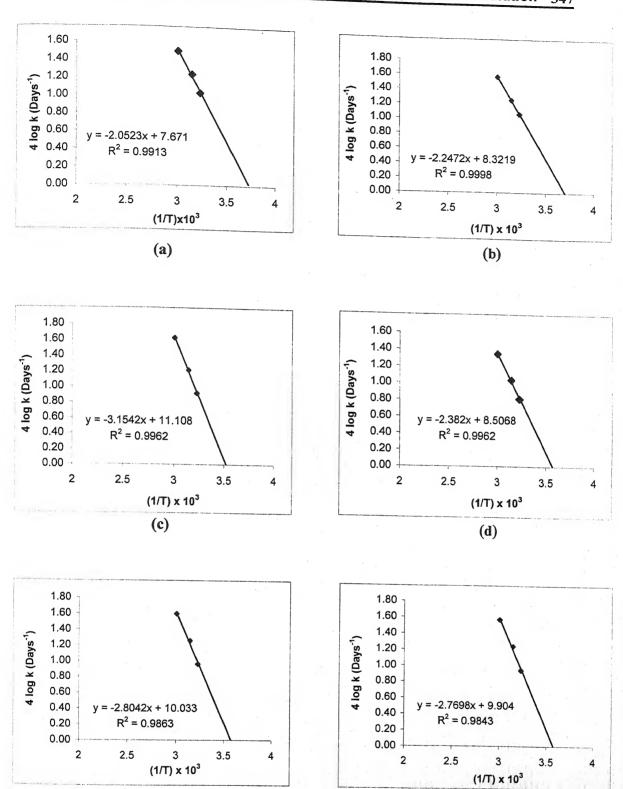
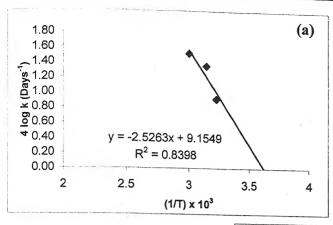
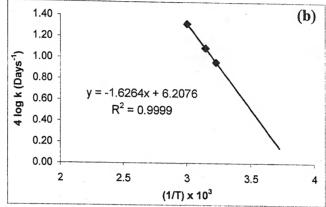


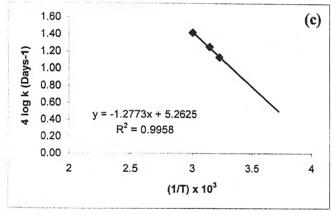
Fig. 5.2.15: Arrehenius plot for (a) I-1a (b) I-1b (c) I-2a (d) I-2b (e) I-3a (f) I-3b formulations

(f)

(e)







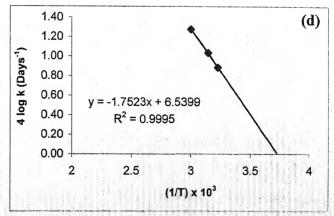


Fig. 5.2.16: Arrehenius plot for (a) I-4 (b) ISB (c) IPHB (d) INMD formulations

5.2.4 Results and Discussion

The physical stability studies of chlorzoxazone formulations showed that except slight colour change in formulation C-2, all the formulations remained unchanged with respect to colour stability. No turbidity or precipitate formation was observed in formulation C-3 at all storage conditions. But formulation C-1 and C-2 showed slight precipitation on 31st day and 39th day respectively when stored in temperature cycling with shaking (TCS) condition, while at room temperature and freezing temperature (4±2°C) in dark there was no precipitate formation upto 45 days. The pH of all the formulations remained within the specified limit upto 45 days (Table 5.2.1).

The physical stability studies of indomethacin formulations showed that except slight colour change in formulation I-1b, I-6 and IPHB, all the formulations remained unchanged with respect to colour stability. No turbidity or precipitate formation was observed in formulation I-2b, I-3a, I-3b, I-4 at all storage conditions. But formulation I-1a, I-1b, I-2a, ISB, IPHB and INMD showed slight precipitation on 13th, 19th, 43rd, 41st, 14th, and 42nd day respectively when stored in temperature cycling with shaking (TCS) condition and I-1a, I-1b and IPHB showed precipitation on 31st, 39th and 31st day respectively when stored at freezing temperature in dark. At room temperature in dark there was no precipitate formation upto 45 days in all the ten formulations. The pH of all the formulations remained within the specified limit upto 45 days (Table 5.2.2).

The selected formulations were stored at 37±2°C, 45±2°C and 60±2°C and the residual drug content of formulation was measured after 1, 3, 7, 14, 21, 30 and 45 days.

For chlorzoxazone formulations the percent residual drug content of selected formulation is presented in table 5.2.3-5.2.5. The plot of log % residual drug vs. time (days) was found to be straight line, which indicates the degradation, follows first order kinetics (Fig. 5.2.1-5.2.3). The rate of degradation of formulations were faster at elevated temperatures. The degradation rate constants at elevated temperature were obtained by the

slope of log% residual drug vs. time (days) plot and shown in table 5.2.6. The degradation rate constant at 25°C (RT) were obtained by the extrapolation of Arrhenius plot (Fig. 5.2.4) and found to be minimum for C-3 formulation i.e. 8.33, intermediate for C-2 i.e. 9.15 and maximum for C-1 i.e. 9.76 days⁻¹x10⁴ at 37±2°C.

The shelf life of formulations were calculated from the degradation rate constant at 25°C and found to be 175.083, 163.677 and 211.273 days for formulation C-1, C-2 and C-3 respectively at 25±2°C as shown in table 5.2.6. Thus among the three formulations C-3 formulation was found to be most stable.

For indomethacin formulations the percent residual drug content of selected formulation is presented in table 5.2.7-5.2.16. The plots of log% residual drug vs. time (days) for all formulations of indomethacin were constructed to determine the degradation rate constants (Fig. 5.2.5-5.2.14) at elevated temperatures. The degradation rate constant at 25°C of the all formulations were obtained by extrapolation of Arrhenius plot (Fig. 5.2.15 and 5.2.16). Among these formulations of indomethacin the degradation rate constant (25°C) was found to be lowest for the formulation I-2b that was 6.47 days⁻¹x10⁴ at 37±2°C. The shelf life of formulations were found to be 171.0, 172.2, 311.6, 318.8, 247.4, 255.7, 218.6, 185.1, 109.8 and 227.7 days for formulation I-1a, I-1b, I-2a, I-2b, I-3a, I-3b, I-4, ISB, IPHB and INMD respectively at 25±2°C as shown in table 5.2.17. Thus among all the ten formulations I-2b formulations was found to be most stable. Among cosolvent formulations, formulations containing higher concentration of cosolvent were found to be more stable i.e. I-1b, I-2b, I-3b. Among formulation containing hydrotropes, the formulation INMD (with nicotinamide) was found to be most stable.

IN VITRO STUDIES 5.3

In vitro testing of formulation was done for evaluating the effects of dilution during injection and to study the haemolytic behavior of formulations and additives that were used for solubilization.

5.3.1 Precipitation (by Dilution Method) Studies

For drug to be therapeutically active the concentration at the site of administration should exceed its aqueous solubility. Precipitation of drug upon injecting a solubilized formulation into fluids often occurs²⁷⁻²⁹. This ultimately results in poor patient compliance. The amount of precipitation can be correlated with the rate at which drug is injected. Any remedies, which reduce or eliminate precipitation, ensure more efficacious formulation. In vitro method for determination of such effect is dilution of formulation with intravenous fluid³⁰

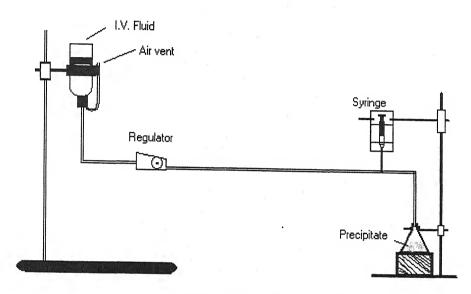
Method

The effect of dilution with intravenous fluids (normal saline/5% dextrose solution) was studied on selected formulation of chlorzoxazone namely C-1, C-2 and C-3 and formulation of indomethacin namely I-1b, I-2b, I-3b and I-4.

Test dilutions of solutions of indomethacin and chlorzoxazone (formulated products) in different vehicles of different concentrations were prepared in thoroughly cleaned and dried volumetric flasks with normal saline and 5% dextrose solution. Dilutions were made in duplicate at 25±1° C. The prepared dilutions (1:1 to 1:50) were examined visually against black and white background using a magnifying glass for the presence of visible precipitate or micro crystal using a sample of intravenous fluid for comparison. The results are shown in table 5.3.1-5.3.7.

In a parallel study, the dilution effect, simulating the flow of i.v. fluids in the body, was studied. The apparatus used in this study is shown schematically in the figure given below. It was designed to provide a reasonable simulation of the events that occur when a drug formulation is injected into the venous system, or into a intravenous drip tube.

The inlet air pressure provided flow of aqueous (normal saline or 5% dextrose solution) at a rate of 20ml/min through a polyethylene tube (2mm i.d.). Formulated aqueous solutions (drug-vehicle mixture) were injected into the tubing through a 22-gauze needle. The rate of injection varied from 0.5 to 2.0 ml/min and the solutions were inspected visually for the appearance of microcrystals.



Schematic diagram of the apparatus used to detect precipitation

Table 5.3.1: Precipitation of chlorzoxazone formulation C-1 after dilution with normal saline or 5% dextrose solution

			Tin	ne (Hi	r) for F	Precip	itatio	n to oc	cur a	fter Dil	ution	with	
S. No.	Dilution		N	lorma	ıl salir	ne	*	*	5% [extro	se Sol	ution	
		0.5	1	2	3	6	24	0.5	1	2	3	6	24
1	1:1	+	+	+	+	+	+	+	+	+	+	+	+
2	1:2	+	+	+	+ -	+	+	+	+	+	+	+	+
3	1:5	+	+	+	+	+	+	+	+	+	+	- +	+
4	1:10	+	+	+	+ 1	+	-	+	+	+	+	+	+
5	1:20	-	+	+	+	+	-	+	+	+	+	+	+
6	1:30	-	+	+	+	+	_		-	-	-	+	+
7	1:50	-	-	-	_	+	-		-	_	-	-	_

⁺ Indicates presence of Precipitate

⁻ Indicates absence of Precipitate

Table 5.3.2: Precipitation of chlorzoxazone formulation C-2 after dilution with normal saline or 5% dextrose solution

			Tin	ne (Hr) for F	recip	itatio	1 to oc	cur at	ter Dil	ution	with	
S. No.	Dilution		Normal saline							extro			
		0.5	1	2	3	6	24	0.5	1	2	3	6	24
1	1:1	+	+	+	+	+	+	+	+	+	+	+	+
2	1:2	+	+	+	+	+	+	+	+	+	+	+	+
3	1:5	+	+	+	+	+	+	+	+	+	+	+	+
4	1:10	-	-	+	+	+	_	_	_	+	+	+	+
5	1:20	-	_	_	+	+	_	_	_	_	+	+	+
6	1:30	_	_	_	· •	_	_	_	_	_			+
7	1:50	_	-	_	_		_	_	_	_	_	_	_

⁺ Indicates presence of Precipitate

Table 5.3.3: Precipitation of chlorzoxazone formulation C-3 after dilution with normal saline or 5% dextrose Solution

			Tin	ne (Hr) for F	Precip	itatio	ı to oc	cur at	ter Dil	ution	with			
S. No.	Dilution	Normal saline							5% Dextrose Solution						
		0.5	1	2	3	6	24	0.5	1	2	3	6	24		
1	1:1	+	+	+	+	+	+	+	+	+	+	+	+		
2	1:2	+	+	+	+	+	+	+	+	+	+	+	+		
3	1:5	+	+	+	+	+	+ -	+	+	+	+	+	+		
4	1:10	_	+	+	+	+	*	_	_	+	+	+	+		
5	1:20	-	-	+	+	+	_	-	-	_	-	+	+		
6	1:30	- 9	-	-	-	_	_	-	_	-	_	-	+		
7	1:50	-	-	_		-		· · <u>·</u> -	_	_	_	_			

⁺ Indicates presence of Precipitate

⁻ Indicates absence of Precipitate

⁻ Indicates absence of Precipitate

Table 5.3.4: Precipitation of indomethacin formulation I-1b after dilution with normal saline or 5% dextrose solution

		Time (Hr) for Precipitation to occur after Dilution with									with			
S. No.	Dilution							5% Dextrose Solution						
		0.5	1	2	3	6	24	0.5	1	2	3	6	24	
1	1:1	-	+	+	+	+	+		+	+	+	+	+	
2	1:2	-	-	+	+	+	+	_	+	+	+	+	+	
3	1:5	_	-	-	+	+	+	-	_	+	+	+	+	
4	1:10	-	_	_		_	_	_	_	_	_	· × _		
5	1:20	-	_	_	_	_	_	_	_	_				
6	1:30	_	_	_	_	_	_	_	_	_	_	_	-	
7	1:50	-	-	-	-	_	-	_	_	_	_	-	-	

⁺ Indicates presence of Precipitate

Table 5.3.5: Precipitation of indomethacin formulation I-2b after dilution with normal saline or 5% dextrose solution

_		Time (Hr) for Precipitation to occur after Dilution with									with	-	
S. No.	Dilution		N	orma	Salin	ie		5% Dextrose Solution					
		0.5	1	2	3	6	24	0.5	1	2	3	6	24
1	1:1	-	-	+	+	+	+	-	+	+	+	+	+
2	1:2	-	-		+	+	+	-	+	+	+	+	+
3	1:5	-	. *-	-	_	+ "	+	_	-	+	+,.	+	+
4	1:10	-	-	_	_		_	-	<u>-</u>	_	-	_	+
5	1:20	-	-	-	_	-	-	-	_	-	_		
6	1:30	-	-	_	_				100	· -	<u> -</u>) <u>-</u>	-
7	1:50	-*	-	×* <u>-</u>	_	_			<u> </u>	-	-	-	_

⁺ Indicates presence of Precipitate

⁻ Indicates absence of Precipitate

⁻ Indicates absence of Precipitate

Table 5.3.6: Precipitation of indomethacin formulation I-3b after dilution with normal saline or 5% dextrose solution

	Time (Hr) for Precipitation to occur after Dilution w										with			
S. No.	Dilution			lorma					5% Dextrose Solution					
		0.5	1	2	3	6	24	0.5	1	2	3	6	24	
1	1:1	-	_	+	+	+	+	-	-	+	+	+	+	
2	1:2	-	-	-	+	+	+	-		+	+	+	+	
3	1:5	-	_	-	-,	+	+	-	_	-	_	+	+	
4	1:10	-	-	-	-	-	_	-	_	_	_	_	-	
5	1:20	-	-		_	-	_	_	-	-	-	_	_	
6	1:30	-	-	-		,_	_	-	· _	_	-	_	_	
7	1:50	-	-	-	-	-	-	-	-	_	-	_	_	

⁺ Indicates presence of Precipitate

Table 5.3.7: Precipitation of indomethacin formulation I-4 after dilution with normal saline or 5% dextrose solution

	,	Time (Hr) for Precipitation to occur after Dilution with												
S. No.	Dilution		Normal saline						5% Dextrose Solution					
		0.5	1	2	3	6	24	0.5	1	2	3	6	24	
1	1:1	-	+	+	+	+	+	-	+	+	+	+	+	
2	1:2	_	_	+	+	+	+	-	+	+	+	+	+	
3	1:5	-	_	_	- "	+	+	<u>.</u>		+ ,	+	+	+	
4	1:10	_	_	-		* -	+					· ·	+	
5	1:20	-	× <u>-</u>	_ =	* <u>-</u> ,	· · ·	- -	-	_	1.	<u> -</u> -	- "	, . .	
6	1:30	-			1	(-1	-	Mag.	-		·	· •	-	
7	1:50	-	-	NE.		,	+ 17		-	711			0./7	

⁺ Indicates presence of Precipitate

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⁻ Indicates absence of Precipitate

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5.3.2 Haemolysis Studies

The drug additives required to design a dosage form may have haemolytic effect³¹⁻³³. Hence, haemolytic studies were carried out to on these additives that were used for solubilization and formulation development and to the formulation³⁰.

Method

The drug as well as additives in parenteral preparations may have a haemolytic effect on the RBCs because of the difference in cellular activity against the erythrocytes. The vehicles may be hypotonic or hypertonic in nature, and may result in the lysis of RBCs. Hence, the haemolytic studies were carried out for the selected formulations of chlorzoxazone namely C-1, C-2 and C-3 and formulations of indomethacin namely I-1b, I-2b, I-3b and I-4. The rabbit blood used in this study was obtained from the marginal ear vein of a healthy male rabbit (mixed strain, 3.07 kg). The blood was collected and immediately difibrinated by gentle rotation in a glass flask with glass beads, until the fibrin had separated. The difibrinated blood was poured into a small glass flask and aerated by gently swirling the flask for about 5 min. After defibrination, the RBCs were washed with normal saline and centrifuged until the supernatant was colourless. The RBCs were then diluted to original volume (2ml) with normal saline solution.

Different concentrations of indomethacin and chlorzoxazone were obtained separately by diluting the formulations with normal saline. A colorimetric method³⁴ was employed to determine the degree of haemolysis in each test solution. 10 ml of these solutions were incubated with 0.1 ml of RBCs suspension at 25±1°C for 45 min. The un-haemolyzed cells were separated by centrifugation at 3000 rpm for 10 min and the absorbance readings of the haemolysate were noted at 550 nm. Each absorbance reading was compared with a total haemolysis reading obtained by taking RBCs in water (1:10). The degree of haemolysis is occurring in each test solution was calculated as a percent of total haemolysis. These results are plotted 5.3.1 and 5.3.2.

Similarly, experiments were carried out to test the haemolytic activity of different concentrations of solubilizers (hydrotrope solution as well as cosolvent blends) used in selected formulations at different sodium chloride concentrations (0.45 - 1.8% w/v). The results are plotted in figure 5.3.3-5.3.9.

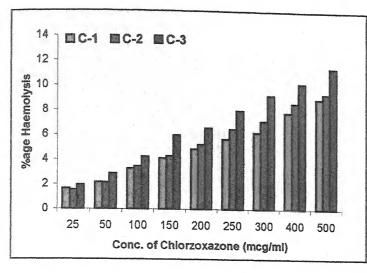


Fig. 5.3.1: Haemolytic **Activity of Chlorzoxazone** Formulation

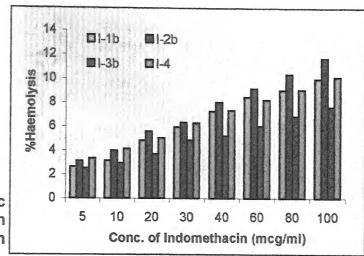


Fig. 5.3.2: Haemolytic Activity of Indomethacin Formulation

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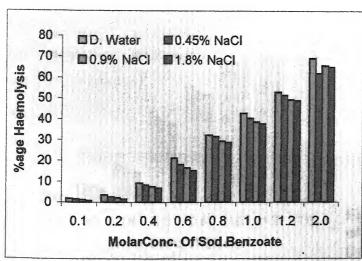


Fig. 5.3.3: Haemolytic Activity of Sod.Benzoate at Different **NaCl Concentration**

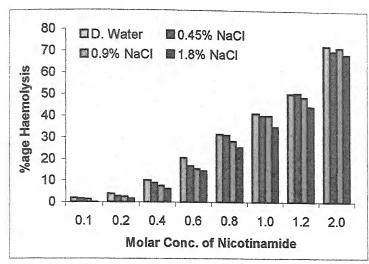


Fig. 5.3.4: Haemolytic Activity of Nicotinamide at Different **NaCl Concentration**

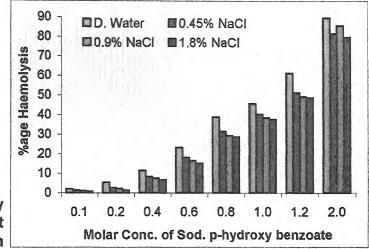


Fig. 5.3.5: Haemolytic Activity of Sod.p-hydroxy benzoate at Different NaCl Concentration

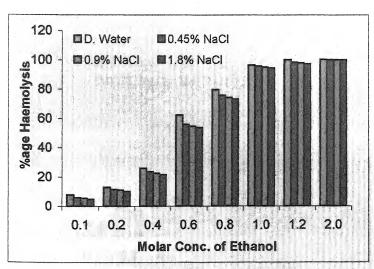


Fig. 5.4.6: Haemolytic Activity of Ethanol at Different NaCl Concentration

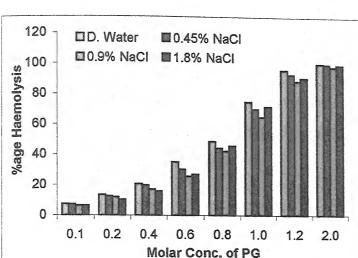


Fig. 5.3.7: Haemolytic Activity of PG at Different NaCl Concentration

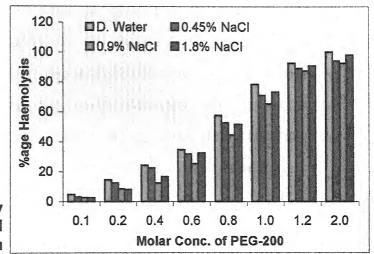


Fig. 5.3.8: Haemolytic Activity of PEG-200 at Different NaCl Concentration

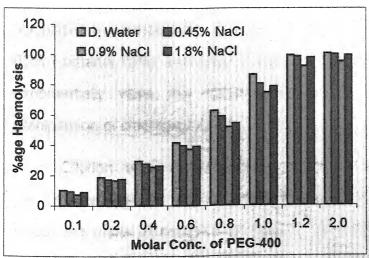


Fig. 5.3.9: Haemolytic Activity of PEG-400 at Different NaCl Concentration

5.3.3 Results and Discussion

On the basis of the result of physical and chemical stability testing, the three promising formulations of chlorzoxazone namely C-1, C-2 and C-3 and four promising formulation of indomethacin namely I-1b, I-2b, I-3b and I-4 were selected for *in vitro* evaluation.

The administration of any parenteral formulation via the intravenous route may result in the precipitation of drug on dilution with plasma or i.v. fluids. The selected formulations were studied for the effect of dilution with normal saline or 5% dextrose solution. The serial dilution of each formulation were prepared in the ration of 1:1 to 1:50 with normal saline or 5% dextrose solution and examined visually for the appearance of precipitate or microcrystals. In a parallel study, the dilution effect was studied, simulating the flow of i.v. fluids in the body, using a fabricated apparatus. The flow rate of aqueous fluid (normal saline/5% dextrose solution) was maintained at 20ml/min while the rate of injection was varied from 0.5 to 2.0 ml/min.

Chlorzoxazone formulations when diluted with normal saline or 5% dextrose solution showed immediate precipitation in all the formulations (Table 5.3.1-5.3.3), however, in higher dilution ratio, the precipitate was redissolved. In these formulations, high concentration of chlorzoxazone was solubilized by incorporation of higher cosolvent concentration (PEG-400 upto 80% + ethanol 10%), thus after dilution, the solubility of chlorzoxazone reduce exponentially while the volume increase linearly. Hence, resulted in precipitation of chlorzoxazone.

Dilution of a formulation of indomethacin with normal saline or 5% dextrose solution did not result in immediate precipitation of transient cloudiness under all condition of dilution (Table 5.3.4-5.3.7). The observation reveal that in formulation I-3b all the test remained clear for at least 1 h, while

the other formulation remained clear for about 0.5 h, with normal saline as well as 5% dextrose solution, but slight to clearly visible microcrystalline precipitate was appeared afterwards at lower dilution ratio. All the formulations were observed to have better stability towards precipitate formulation with normal saline compare with 5% dextrose solution. As the dilution ratio was increased, the appearance of precipitate was faster, but after much higher dilution (e.g. 1:30 to 1:50), the precipitate partly disappeared. This might be due to the redissolution of precipitate.

All the selected formulations were subjected to in vitro haemolytic studies using red blood cells (RBCs) of rabbit blood. The degree of hemolysis was estimated by a colorimetric method34, which is free from interference of oxyhaemoglobin contents at 550 nm. The haemolytic activity of chlorzoxazone (Fig. 5.3.2) and indomethacin (Fig. 5.3.3) in different formulations at different drug concentrations was studied. The data clearly shows that all the selected formulations exhibit a haemolytic effect. The formulation of both the drugs with 500 µg/ml drug concentration resulted in little, nearly 11% haemolysis. Among chlorzoxazone formulations haemolytic behaviour can be ranked as C-3> C-2>C-1 and among the formulations of indomethacin, the haemolytic behaviour can be ranked as I-2b>I-4>I-1b>I-3b.

The hydrotropes were also individually evaluated in the concentration range 0.2-2 M to determine their haemolytic potential. The results shows that all the hydrotropes studied exerted a negligible haemolytic effect at concentration below 0.4 M but highly significant haemolysis above 0.4 M of the hydrotrope concentration. All the hydrotrope produces nearly 80% haemolysis when studied in water in the concentration of 2 M (Fig. 5.3.4-5.3.6). The haemolytic activity of these hydrotropes can be ranked as Sodium p-hydroxy benzoate > nicotinamide > sodium benzoate. The different concentration of cosolvents were also individually evaluated to determine their

haemolytic activity. Cosolvents PG, PEG-200 and PEG-400 showed about 100% haemolysis when used in concentration above 60%, while ethanol showed about 100% haemolysis when used in concentration above 10% (Fig. 5.3.7-5.3.10). Thus the haemolytic activity of these cosolvents can be ranked as ethanol>PEG-400>PEG-200>PG.

In a parallel study, the effect of varying concentration of sodium chloride (0.45-1.8%) on haemolytic behaviour of the hydrotrope in concentration range 0.2-2 M and upto 70% of cosolvents was also studied. Sodium chloride at the 1.8% concentration significantly reduced the haemolytic activity of sodium benzoate, nicotinamide, sodium p-hydroxy benzoate and ethanol, while in case of PG, PEG-200 and PEG-400 0.9% NaCl reduces the haemolytic activity significantly. It is important to note that the in vitro haemolysis in the present study would be unlikely to occur in vivo, as the intravenously injected solution will be diluted about 25 fold in the total blood volume.

5.4 IN VIVO STUDIES

In vivo studies are important to evaluate the physical availability of drug from a dosage form. These types of studies generally performed by either observing pharmacological response or by constructing drug-plasma time profile and determining different pharmacokinetic parameters using a suitable animal model. In the present study the bioavailability of the promising formulation of chlorzoxazone and indomethacin was compared with the standard marketed formulation or oral dose of the drug by constructing drugplasma time profile.

5.4.1 Pharmacokinetics

The primary goals of pharmacokinetics are to quantify drug absorption distribution, biotransformation and excretion in the intact, living animal or man; and to use this information to predict the effect to alterations in the dose, dosage regimen, route of administration and disposition. pharmacokinetics of a drug can be deduced by studying the time courses of changes in drug or metabolite concentrations in body fluids. The drug concentration in plasma or urine at any time after the administration of a known dose or dosage regimen is the net result of its absorption distribution, biotransformation (metabolism) and excretion. The task therefore, is to resolve the observed kinetic profiles into their component parts. The contribution of absorption, distribution, biotransformation and excretion can be individually isolated by appropriate experimental design and kinetic analysis of the data, often with the aid of models. Quantitation is then achieved by maintaining material balance at all times.

Pharmacokinetic Studies

Based on the stability studies and in vitro evaluation, four formulation of indomethacin namely I-1b, I-2b, I-3b and I-4 and three formulation of chlorzoxazone namely C-1, C-2b and C-3b were selected for pharmacokinetic studies in rabbit.

5.4.2 Method of Estimation of Drug

5.4.2.1 Indomethacin

A modified HPLC - UV method, as reported by Notarianni and Collins³⁵, Zones et al.³⁶, Hu et al.³⁷ and Casella et al.³⁸ was used to determined Indomethacin. Briefly Jasco HPLC System Consisted of JASCO PU 980 isocratic pump and Jasco UV 975 detector set at 254 nm (Jasco Inc. Japan) was used. The mobile phase used was 65 : 35 ratio acetonitrile : water solution. Operating condition were: column 150 X 4.6mm Techsphero Octadecyl Silica (ODS) with 12 X 4.6 mm ODS safe guard column; flow rate 1.0ml/min at room temperature; and injection volume 20 µl; phenylbutazone, was used as an internal standard.

10 mg of indomethacin was accurately weighed and dissolve in methanol and serial standard solution consisting of 0, 0.1, 0.2, 0.4, 0.6,0.8, 1.0, 1.2 & 1.5 µg/ml. of indomethacin were prepared respectively. Aliquots of Indomethacin 50 µl and internal standard solutions (phenylbutazone in methanol) 50 µl were added to blank plasma 0.4 ml in a 10ml screw capped culture tube, and then mobile phase solution 0.2 ml was added. The mixed solution was mixed for 30 sec using vertex mixer. 1,2 Dichloroethane 5 ml was added to the tube, the tube was vibrated for 10 min using a vibrator and then centrifuged at 4000 rpm for 20 min. The organic layer was transferred to another tube and evaporated in a 45°C water bath. The residue evaporated was reconstituted use in mobile phase solution 100 µl and injected into HPLC system.

The lower limit of quantification (LOQ) of the assay were 0.01 µg/ml for indomethacin when the signal to noise ratio were set at 3. Linearity was obtained between 0.1 μ g/ml and 1.5 μ g/ml (r ² = 0.9993).

The essay method was validated according to a method reported by Brooks and Weinfeld³⁹. The indomethacin calibration curve (n = 3) recover from rabbit plasma is shown in figure 5.4.1.

The validation data reported in table 5.4.1. The result shows that the method was linear, precise, accurate and reproducible. The correlation coefficient (r²) was 0.9993, intercept was 0.013 and slope was 1.2314.

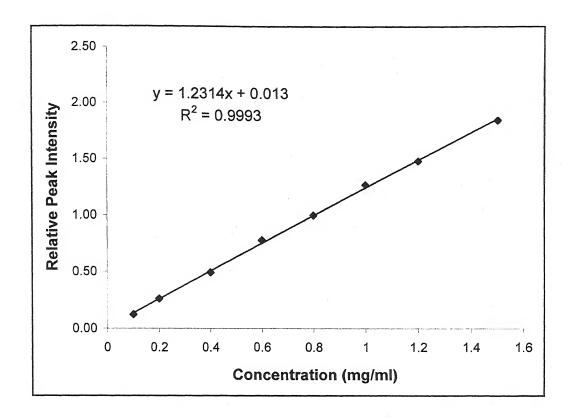


Fig. 5.4.1: Standard curve of indomethacin in rabbit plasma

Table 5.4.1: Validation of Indomethacin assay

Parameter	Value
Linearity (Correlation Coefficient) r ²	0.9993
Recovery	89.4± 4.7%
Slope	1.2314
Intercept	0.013
Quantitation Range	0.1: g/ml to 1.5 : g/ml
Detection Limit	0.01: g/ml (10ng/ml)

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5.4.2.2 Chlorzoxazone

A modified HPLC assay method as reported by Honigberg et al. 40 and Stewart and Carter⁴¹ was used to estimate the Chlorzoxazone level in rabbit plasma. The separation was achieved on an octadecylsilane; (25cm X 4.6 mm i.d.) using a mobile phase of 50: 50 acetonitrile: distilled water with flow rate 2.0 ml/min and UV detection at 280 nm. Phenacetin was used as internal standard. A plasma sample was made acidic with hydrochloric acid and extracted once with ethyl acetate. The ethyl acetate was evaporated to dryness and the residue dissolved in methanol prior to injection into HPLC.

10 mg. of chlorzoxazone was accurately weighed and dissolve in methanol and serial standard solution consisting of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 µg/ml of chlorzoxazone were prepared respectively. Aliquots of chlorzoxazone 50 µl and internal standard solutions (phenacetin in methanol) 50 µl were added to blank plasma 0.4 ml in a 10ml screw capped culture tube, and then mobile phase solution 0.2 ml was added. The solution was acidified with 0.1ml 0.1N hydrochloric acid and mixed for 30 sec using vertex mixer. Ethyl acetate 5 ml was added to the tube, the tube was vibrated for 10 min using a vibrator and then centrifuged at 4000 rpm for 20 min. The organic layer was transferred to another tube and evaporated to dryness at 60°C. The residue evaporated was reconstituted using methanol 100 µl and injected into HPLC system.

The lower limit of quantification (LOQ) of the assay was 0.08 µg/ml for chlorzoxazone when the signal to noise ratio were set at 2. Linearity was obtained between 0.1 μ g/ml and 1.5 μ g/ml ($r^2 = 0.9993$).

The essay method was validated according to a method reported by Stewart and Carter⁴¹. The chlorzoxazone calibration curve (n = 3) recover from rabbit plasma is shown in figure 5.4.2. The validation data reported in table 5.4.2. The result shown that the method was linear, precise, accurate and reproducible. The correlation coefficient (r2) was 0.9968, intercept was -0.0167 and slope was 1.0605.



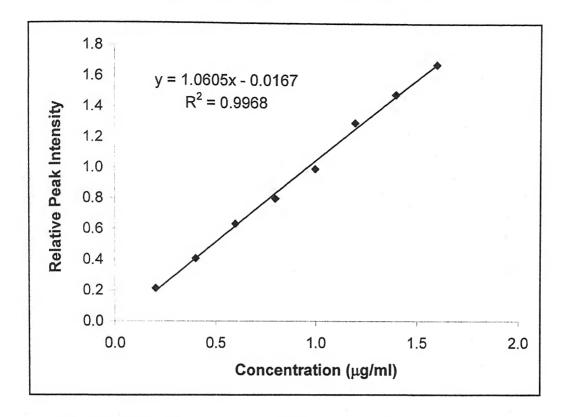


Fig. 5.4.2: Standard curve of chlorzoxazone in rabbit plasma

Table 5.4.2: Validation of Chlorzoxazone assay

Parameter	Value
Linearity (Correlation Coefficient) r ²	0.9968
Recovery	90.24±4.28%
Slope	1.0605
Intercept	-0.0167
Quantitation Range	0.2: g/ml to 1.6 : g/ml
Detection Limit	0.08: g/ml (80ng/ml)

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5.4.3 Blood Plasma Profile of Chlorzoxazone Formulations

5.4.3.1 Drug administration and blood sample collection

Four groups, each of six healthy male rabbits (mixed strain) weighting 3.04±0.05 kg (their weight remained constant throughout the study) were selected for the study. These animals were fasted over night prior to dosing but were allowed access to water. Each animal has placed in body-restrained device, which exposed the animal's head. One ml of blood sample was collected from marginal ear vein, which served as control. The formulations C-1, C-2 and C-3 (20 mg/kg body weight) were injected intravenously in three different groups, each of six rabbits into marginal ear vein and the rabbits were left free in cages.

To the fourth group of sixth male healthy rabbit was administered powder of chlorzoxazone 20mg/kg body weight, using a gastric canula with 20 ml of water. Blood samples were drawn by veinipuncture at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 hrs. Blood samples were taken with animal placed in a body restraint cage that allowed easy access to the ears. Xylene was applied to the shaved marginal ear vein, which caused this blood vessel to dilate. The vein was punctured with a 27 gauge needle and blood was sucked into heparinazed disposable syringe.

5.4.3.2 Preparation of blood sample and assay of chlorzoxazone

Blood samples were centrifuged for 10 minute at about 3000 rpm to obtain plasma and stored in a deep freezer at -20°C until subsequent analysis.

For assay 0.2 ml plasma aliquot was taken and mixed with 0.7 ml of 44:56 ratio of methanol : acetonitrile solution to precipitate plasma proteins additionally 0.1ml of 4.9 X 10⁻⁵ N phenacetin, dissolved in methanol was added to each sample as an internal standard. This mixture was acidified with 0.2ml 0.1N hydrochloric acid and mixed for 30 sec using a vertex mixture. Ethyl acetate 5 ml was added and vibrated for 10 minute using a vibrator and than centrifuged at 4000 rpm for 20 minutes. The organic layer was transferred to another tube and evaporated at 60°C. The residue evaporated was reconstituted using methanol 100 µl and injected into HPLC system. Quantification of chlorzoxazone was done using standard curve equation. The plasma concentration time profiles are shown in table 5.4.3 and graphically presented in figure 5.4.3. Semilogarithmic plots of drug plasma concentration vs. time (Fig. 5.4.4) were plotted to obtain the elimination rate constant. Other pharmacokinetic parameters were also calculated and shown in table 5.4.5.

Table 5.4.3: Plasma chlorzoxazone concentration (mcg/ml) from selected formulation with 20 mg\kg dose

S.No.	Time	Plasma Chlorzoxazone Concentration (mcg/ml) from selected formulaion with 20mg\kg dose									
	_	C-1	C-2	C-3	CLZ						
1	0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00						
2	0.25	44.56±6.82	43.38±6.45	42.86±6.17	2.28±0.35						
3	0.50	39.01±5.94	36.72±5.97	37.24±5.84	5.67±0.94						
4	0.75	33.76±5.24	30.58±4.86	31.28±5.51	12.34±1.46						
5	1.00	31.29±5.34	27.86±5.12	29.23±5.02	23.49±3.16						
6	1.50	28.21±5.64	25.16±4.32	27.53±4.76	34.56±5.21						
7	2.00	25.91±4.25	22.59±4.15	23.19±4.29	30.43±4.83						
8	3.00	19.57±3.40	18.24±3.42	17.98±3.17	19.25±3.43						
9	4.00	14.52±2.90	13.57±2.71	13.04±2.34	12.87±2.14						
10	6.00	9.21±1.54	8.84±1.28	9.14±1.33	10.04±1.29						
11	8.00	4.26±0.85	4.16±0.83	4.35±0.87	4.51±0.78						
12	12.00	1.83±0.37	1.74±0.35	1.89±0.38	2.37±0.39						

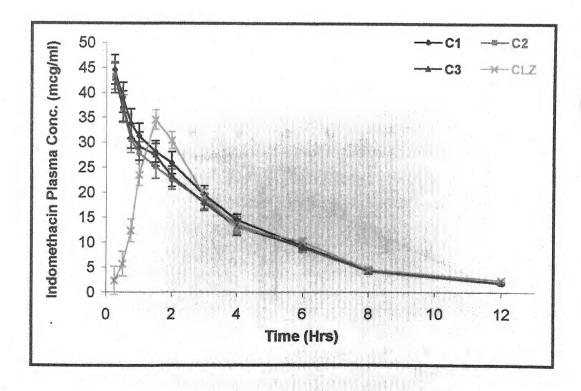
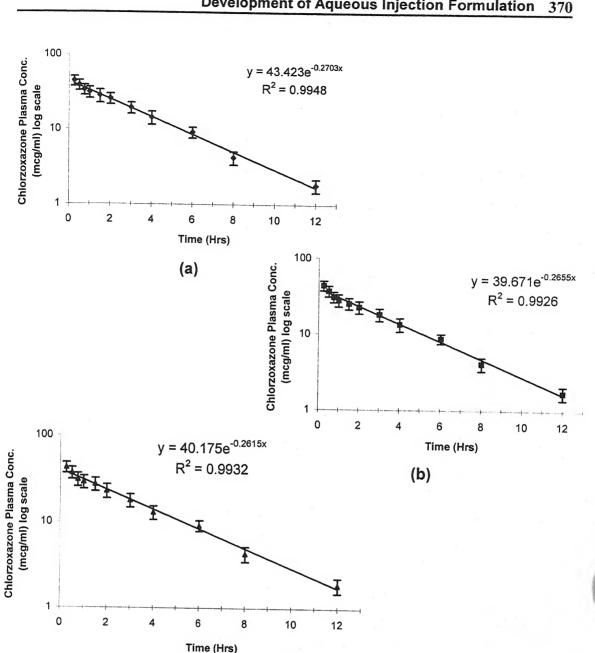


Fig. 5.4.3: Chlorzoxazone-plasma profile



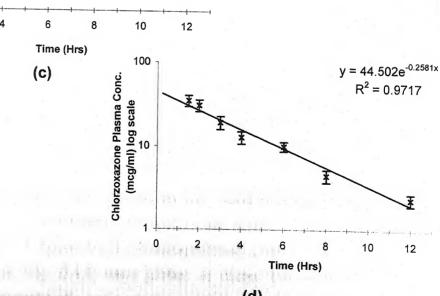


Fig 5.4.4: Plot for determination of elemination rate (a) C-1 (b) C-2 (c) C-3 and (d) Chlozoxazone oral formulation

5.4.4 Drug Plasma Profile of Indomethacin Formulation 5.4.4.1 Drug administration and blood sample collection

After a wash out period of one week, above five groups each of six male healthy rabbits (mixed strain) with an average weight of 3.04 \pm 0.05 kg were used for the study. The animals were fasted overnight prior to dosing but were allowed access to water. Each animal has placed in body-restrained device, which exposed the animal's head. One ml of blood sample was collected from marginal ear vein, which served as control. The formulations I-1b, I-2b, I-3b and I-4 (5mg/kg body weight) were injected intravenously in four different groups, each of six rabbits into marginal ear vein and the rabbits were left free in cages.

To the fifth group of sixth male healthy rabbit was administered powder of proprietary capsule (Idicin-IDPL) equivalent to 50mg/kg body weight of indomethacin, using a gastric canula with 20 ml of water. Blood samples were drawn by veinipuncture at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 hrs. Blood samples were taken with animal placed in a body restraint cage that allowed easy access to the ears. Xylene was applied to the shaved marginal ear vein, which caused this blood vessel to dilate. The vein was punctured with a 27 gauge needle and blood was sucked into heparinazed disposable syringe.

5.4.4.2 Preparation of blood sample and assay of indomethacin

Blood samples were centrifuged for 10 minute at about 3000 rpm to obtain plasma and stored in a deep freezer at -20°C until Subsequent analysis.

For assay 0.2 ml plasma aliquot was taken and mixed with 0.9 ml. of 44:56 ratio of methanol; acetonitrile solution to precipitate plasma proteins additionally 0.1ml of 4.9 X 10⁻⁵ N Phenylbutazone, dissolved in methanol was added to each sample as an internal standard. This mixture was mixed for 30 sec using a vertex mixture. 1,2, dichloroethane (5 ml) was added and vibrated for 10 minute using a vibrator and than centrifuged at 4000 rpm for 20 minutes. The organic layer was transferred to another tube and evaporated in a 45°C water bath. The residue evaporated was reconstituted using mobile phase solution 100 µl and injected into HPLC system. Quantification of indomethacin was done using standard curve equation. The plasma concentration time profiles are shown in table 5.4.4 and graphically presented in figure 5.4.5. Semilogarithmic plots of drug plasma concentration vs. time (Fig. 5.4.6) were plotted to obtain the elimination rate constant. Other pharmacokinetic parameters were also calculated and shown in table 5.4.5.

Table 5.4.4: Plasma indomethacin concentration (mcg/ml) from selected formulation with 5 mg\kg dose

S.No.	Time	Plasma Indomethacin Concentration (mcg/ml) from selected formulaion with 5mg\kg dose									
		I-1b	I-2b	I-3b	1-4	Idicin(IDPL)					
1	0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00					
2	0.25	13.04±2.95	12.97±2.75	12.56±2.98	13.18±2.84	0.41±0.10					
3	0.50	12.63±3.02	12.45±2.80	12.17±3.04	12.55±2.46	0.95±0.24					
4	0.75	12.01±2.97	11.89±2.64	11.74±2.47	11.93±2.31	1.46±0.37					
5	1.00	11.12±2.54	10.94±2.21	10.82±2.84	10.71±2.18	2.26±0.57					
6	1.50	10.03±2.16	9.98±2.37	9.79±2.24	9.57±1.97	3.21±0.80					
7	2.00	8.84±2.24	8.72±2.04	8.76±2.03	8.51±1.67	4.49±1.12					
8	3.00	7.54±1.76	7.51±1.52	7.42±1.59	7.79±1.41	4.93±1.13					
9	4.00	5.81±1.12	5.86±1.64	5.48±1.64	5.99±1.16	7.02±1.56					
10	6.00	3.57±0.41	3.42±0.81	3.14±0.74	3.84±0.84	4.59±1.15					
11	8.00	2.38±0.53	2.31±0.49	2.05±0.62	2.52±0.51	4.82±1.06					
12	12.00	0.86±0.21	0.74±0.16	0.77±0.14	0.89±0.24	0.94±0.24					

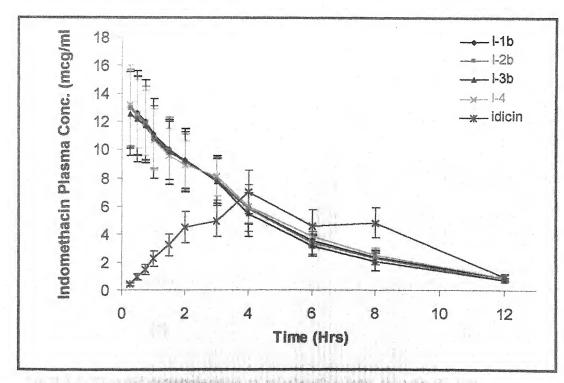


Fig. 5.4.5: Indomethacin-plasma profile

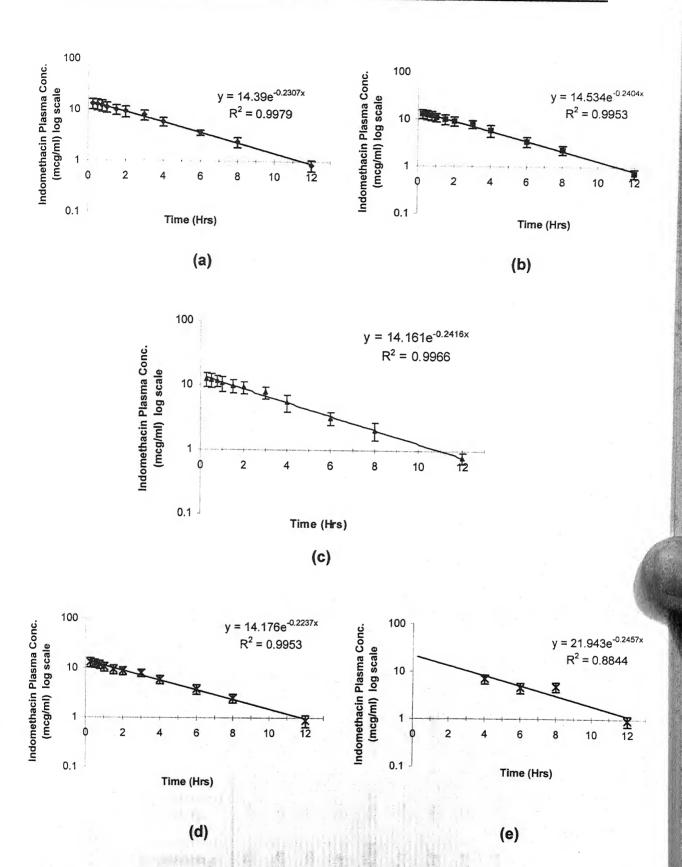


Fig 5.4.6: Plot for determination of elimination rate (a) I-1b (b) I-2b (c) I-3b (d) I-4 and (e) Idicin formulation

	able 5.4	Png	armacokine	etic da	lable 5.4.5: Pharmacokinetic data following administration of indomethacin or chlorzoxazone formulation in rabbit	Idministratio	n of indomet	hacin or ch	lorzoxazone	formulation	n rabbit
'on 's	Formulation	Poute of noistration	C _{max} (: g/ml)	(11) xsmT	Elimination rate constant K (hr ⁻¹)	Apparent of the office of the	Elimination Half Life t _{1/2} (hr)	Apparent Clearance KV/F (ml/min.)	AUC ₀₋₁₂ (: g .h /ml)	d. g :) ⊶₀ЭUA (lm\	Comparative %age Bioavailability
•	179 179	2	13.04±2.95	0.25	0.231±0.095	1.042±0.541	3.004±1.131	4.01±1.25	55.88±2.53	62.38±2.81	115.8 %
5	H2b	2	12.97±2.75	0.25	0.240±0.078	1.032±0.489	2.883±1.095	4.14±1.41	54.92±2.67	60.46±2.73	113.8 %
က	l-3b	2	12.56±2.98	0.25	0.241±0.084	1.059±0.527	2.868±1.090	4.27±1.39	53.68±2.34	58.61±2.45	111.2 %
4	7	Δ.	13.18±2.84	0.25	0.224±0.079	1.058±0.536	3.098±1.177	3.95±1.19	56.75±2.81	63.37±2.49	117.6 %
ç	IDICIN	Oral	7.02±1.56	4.0	0.246±0.092	ı	2.821±1.072	ı	48.26±2.18	1	100.0 %
မှ	5	2	44.56±6.82	0.25	0.270±0.102	1.382±0.493	2.564±0.974	6.23±1.86	151.49±7.26	160.65±6.87	117.8 %
7	C-2	A	43.38±6.45	0.25	0.266±0.118	1.512±0.429	2.610±0.992	6.69±2.07	141.58±6.53	149.42±5.92	110.1 %
∞	င်	>	42.86±6.17	0.25	0.261±0.099	1.493±0.455	2.650±1.007	6.51±1.99	146.55±6.34	153.63±6.24	114.0 %
0	CLZ	Oral	34.56±5.21	1.5	0.258±0.107	ı	2.685±1.020	ı	126.60±5.99	,	100.0 %

Table 5.4.6: Test of Significance Level of Variance in Result in Rabbits For Indomethacin and Chlorzoxazone Formulations

S.No.	Formulations	t-Value*	Conclusion**
1	I-1b / IDICIN (Oral)	3.957	Significant
2	I-2b / IDICIN (Oral)	3.289	Significant
3	I-3b / IDICIN (Oral)	4.127	Significant
4	I-4 / IDICIN (Oral)	5.118	Significant
5	I-1b / I-2b	1.957	Not significant
6	I-1b / I-3b	2.131	Not significant
7	I-1b / I-4	1.649	Not significant
8	I-2b / I-3b	1.237	Not significant
9	I-2b / I-4	1.951	Not significant
10	I-3b / I-4	2.864	Significant
11	C-1 / Chlorzoxazone (Oral)	5.825	Significant
12	C-2 / Chiorzoxazone (Oral)	3.229	Significant
13	C-3 / Chiorzoxazone (Oral)	3.876	Significant
14	C-1 / C-2	2.353	Significant
15	C-1 / C-3	1.394	Not significant
16	C-2 / C-3	1.865	Not significant

* n = 6; ** P =0.05, Two tailed test, DF = 10, Table "t" value = 2.228

5.4.5 Results and Discussion

The Plasma concentration time profile for three injectable formations of chlorzoxazone and chlorzoxazone oral powder (Clz) are shown in figure 5.4.3 and data are given in table 5.4.3. The time of maximum plasma concentration of Clz was about 1.5 hours and peak plasma concentration was 34.56 ± 5.21 µg/ml. The time of maximum plasma concentration (T_{max}) for all injectable formulation C-1, C-2 and C-3 were found to be 0.25 hr. and peak plasma concentration (T_{max}) were found T_{max} 0 were found T_{max} 1 were found T_{max} 2 and T_{max} 3 and T_{max} 4 and T_{max} 5 and T_{max} 6 and T_{max} 7 respectively.

The elimination rate constant K and the apparent volume of distribution V were calculated using first order, one compartment open model with mono exponential decay in drug plasma concentration. The rate of change in plasma concentration of drug C is therefore:

Which upon integration yields:

$$C = C_0 e^{-Kt}$$

$$= DN e^{-Kt} \qquad ...(2)$$

At time zero i.e. t=0, the plasma concentration C_0 is equal to the dose D divided by the volume of distribution V.

Experimentally a semilogrithmic plot of C verses t was a straight line. Model parameters K and C_0 were calculated from slope and intercept of its expontially regressed curve (Curve equation $y = A e^{-Kt}$)

$$Log C = log C_0 - K t / 2.303$$
 ...(3)

Apparent volume of distribution was calculated

$$V = FD / C_0 \qquad ...(4)$$

Where F is fraction of dose that is available to systemic circulation.

The area under the curve from time zero to infinity AUC_0^{∞} is obtained by using Eq (5) :

$$AUC_0^{\infty} = FD / VK \qquad ...(5)$$

The half-life was calculated as follows:

$$T = 0.693 / K$$
 ...(6)

Total plasma clearance rate or plasma clearance CL was calculated as follows.

$$CL = D/AUC = KV$$
 ...(7)

The pharmacokinetic data for all the three formulations of chlorzoxazone and Clz oral are shown in table 5.4.5. The elimination rate constant K was calculated by the slope of log drug plasma concentration vs.

time plot (Fig. 5.4.4) and found to be 0.270±0.102, 0.266±0.118, 0.2.61±0.099 and 0.258 ± 0.107 respectively and elimination half-life $t_{1/2}$ were found 2.564 ± 0.974 , 2.610 ± 0.992 , 2.650 ± 1.007 , and 2.685 ± 1.020 respectively for C-1, C-2, C-3 and Clz oral.

Apparent volume of distribution of all the three chlorzoxazone formulation were found to be 1.382 ± 0.493 , 1.512 ± 0.429 , and 1.493 ± 0.455 liters for C-1, C-2 and C-3 respectively. It was calculated from intravenous dose divided by initial drug plasma concentration C_{0} after intravenous drug administration (As the intravenous dose is considered 100% bioavailable). Initial drug plasma concentration C_0 was obtained by the intercept of exponentially regressed semilogrithmic plot of drug plasma concentration verses time, which was found to be 43.423, 39.671 and 40.175 for C-1, C-2 and C-3 respectively.

The AUC₀[∞] and plasma clearance of chlorzoxazone was calculated using equation 5 and 7 and shown in table 5.4.5.

The bioavailability was assessed and compared using drug plasma profile plot between drug plasma concentration verses time. The area under the curve (AUC $_{0-12}$) was obtained by the integration of curve from 0.25 to 12 hrs and found to be 151.49 ± 7.26 , 141.58 ± 6.53 , 146.55 ± 6.34 and 126.60 ± 5.99 for C-1, C-2, C-3 and Clz oral formulation respectively. Thus, in comparison to Clz oral formulation all the three injectable formulation namely C-1, C-2 and C-3 have 117.8%, 110.1%, and 114.0% bioavailability respectively.

All the three injectable formulation have immediate peak plasma concentration T_{max} = 0.25 hrs in comparison to Clz oral, which have T_{max} = 1.5 hrs as shown in figure 5.4.3.

The result indicates that the AUC₀₋₁₂ for all the four injection formulation are nearly the same. The AUC_{0-12} for the capsule was less than that of injection formulations.

To study the significance level for the variation among different formulations, students t test was applied. The t values are given in table 5.4.6. At p = 0.05, the t values is 2.228 for the two-tail t test. It clearly

indicates that the formulations given orally shows significant variation when compared with any of the injection formulations. This suggests that the difference in bioavailability is small but significant.

So, it can be concluded that the developed parenteral formulations have better bioavailability with less variation in pharmacokinetic parameters than the oral dosage form.

The Plasma concentration time profile for four injectable formations of indomethacin and Idicin are shown in figure 5.4.4 and data are shown in table 5.4.5. The profiles for all injectable formulation were comparable but distinct from Idicin capsule. Idicin lacked a smooth profile, which was attributed to prolonged drug absorption due to entrohepatic recirculation of indomethacin.

The time of maximum Plasma concentration of Idicin capsule was about four hours and peak plasma concentration was 7.02±1.56 μg/ml. Other investigators have reported the time of maximum plasma concentration (T_{max}) of indomethacin was 0-2 hrs after administration to a rabbit by intravenous, rectal, or oral suspension dosage form. The time of maximum plasma concentration (T_{max}) for all injectable formulation I-1b, I-2b, I-3b and I-4 was found to be 0.25 hr. and peak plasma concentration (C_{max}) were found 13.04 ± 2.95 , 12.97 ± 2.75 , 12.56 ± 2.98 and 13.18 ± 2.84 respectively.

The pharmacokinetic data for all the four formulations of indomethacin namely I-1b, I-2b, I-3b, I-4 and marketed capsule formulation Idicin were shown in table 5.4.5. The elimination rate constant K was calculated by the slope of log drug plasma concentration vs. time plot (Fig. 5.4.6) and found to be 0.231 ± 0.095 , 0.240 ± 0.078 , 0.241 ± 0.084 , 0.224 ± 0.079 and 0.246 ± 0.092 respectively and elimination half-life $t_{1/2}$ were found 3.004±1.131, 2.883±1.095, 2.866±1.090, 3.098±1.177 and 2.821±1.027 respectively for I-1b, I-2b, I-3b, I-4 and Idicin.

Apparent volume of distribution of all the four indomethacin formulation were found to be 1.042 ± 0.541 , 1.032 ± 0.489 , 1.059 ± 0.527 and 1.058 ± 0.536 liters for I-1b, I-2b, I-3b and I-4 respectively. This calculated from intravenous dose divided by initial drug plasma concentration Co after intravenous drug

administration (As the intravenous dose is considered 100% bioavailable). Initial drug plasma concentration Co was obtained by the intercept of exponentially regressed semilogrithmic plot of drug plasma concentration verses time, which was found to be 14.39 ± 0.089 , 14.534 ± 0.076 , 14.16 ± 0.085 and 14.176±0.078 for I-1b, I-2b, I-3b and I-4 respectively.

The AUC₀ and plasma clearance of indomethacin was calculated using equation 5 and 7 and shown in table 5.4.5.

The bioavailability was assessed and compared using drug plasma profile plot between drug plasma concentration verses time. The area under the curve (AUC $_{0-12}$) was obtained by the integration of curve from 0.25 to 12 hrs and found to be 55.88±2.53, 54.92±2.67, 53.68±2.34, 56.75±2.81 and 48.26±2.81 for I-1b, I-2b, I-3b, I-4 and Idicin formulation respectively. Thus, in comparison to marketed capsule formulation Idicin all the four injectable formulation namely I-1b, I-2b, I-3b and I-4 have 115.8%, 113.8%, 111.2% and 117.6% bioavailability respectively.

All the four injectable formulation have immediate peak plasma concentration T_{max} =0.25 hrs in comparison to Idicin which have T_{max} =4.0 hrs as shown by figure 5.4.5.

The result indicates that the AUC₀₋₁₂ for all the four injection formulation are nearly the same. The AUC₀₋₁₂ for the capsule was less than that of injection formulations.

To study the significance level for the variation among different formulations, students t test was applied. The t values are given in table 5.4.6. At p = 0.05, the t values is 2.228 for the two-tail t test. It clearly indicates that the formulations given orally shows significant variation when compared with any of the injection formulations. This suggests that the difference in bioavailability is small but significant.

So, it can be concluded that the developed parenteral formulations have better bioavailability with less variation in pharmacokinetic parameters than the oral dosage form.

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Summary & Conclusion Solubility enhancement has broad implication in parenteral formulation design. The choice of a method of solubilization is dependent upon how efficiently the drug can be solubilized and upon the biocompatibility of the vehicle.

Drug solubilization has been a subject of many scientific articles and textbooks; yet despite this attention and available literature, product development scientist still encounters significant difficulties in their solubility problem. Theories of solute solubilization are not easy to understand. Solubilization processes are amazing, complex and require a fair amount of expertise in physical chemistry to interpret and apply current theoretical models. In order to understand what method to utilize for increasing the solubility of a poorly water-soluble drug, it is necessary to understand what is solution and solubilization and why a compound is insoluble.

Water insoluble drug delivery system can be classified in two broad categories.

A. Monophasic solution

Solubilization by pH control or salt form

Solubilization with organic co solvent

Solubilization with surfactant

Solubilization with hydrotropes

Solubilization by complexation

B. Multiphasic dispersed system

Emulsion

Suspension

Liposomes

Nanoparticles

Insoluble drug delivery technology.

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The choice of solubilizing technique depends on many factor: the physico chemical properties of the drug molecules, the desired concentration, the effectiveness of the method, safety and cost of solubilizing agents and formulation related problems associated with intravenous drug delivery. Among these techniques, cosolvency and complexation are highly effective for non-polar drugs. Aqueous solubilization can also be achieved via addition of hydrotropic agents. Hydrotropy is the solubilization process where by addition of a large amount of second solute results in increase in the aqueous solubility of another solute. Cosolvency is aqueous solubilization of poorly soluble compounds in water miscible organic solvents.

The work was envisaged to explore and assess the possibility of employing hydrotropic solubilization, cosolvency, surfactant solubilization (micellization) and complexation to solubilize poorly water insoluble drugs and develop their aqueous injection dosage form. The investigation focuses on insoluble character of drugs, namely chlorzoxazone and indomethacin.

The work undertaken may be summarized under these distinct headings for better under standing:

- 1. Preformulation studies
- 2. Solubilization of drugs
- 3. Formulation of injection
- 4. Biological evaluation.

Extensive literature survey provides useful information, which assisted in the selection of drugs as well as logical development and evaluation of dosage form. Two poorly water-soluble drugs were selected as model for the study: Chlorzoxazone, a centrally acting muscle relaxant and indomethacin. an anti-inflammatory drug.

The gift sample of chlorzoxazone and indomethacin was obtained from M/s Signa Pharma Pvt. Ltd., Kanpur (U.P.), India. Both the drugs were identified and characterized.

Physical appearance and meting point of the drug sample under investigation were found to be concordant with the reported values. Particle

size of both drugs was determined microscopically using calibrated ocular micrometer and found to be $83.48\pm9.36~\mu m$ and $68.23\pm11.56~\mu m$ for chlorzoxazone and indomethacin respectively. Hygroscopicity of the drugs was determined at various humidity conditions in sealed desiccators of well-defined humidity conditions. Both the drugs were classified as non-hygroscopic, because of negligible amount of moisture was gained by drugs when kept at RH below 90% and total moisture gained was less than 20% when stored at RH 90% or above 90% for one week.

Lipophilicity of chlorzoxazone and indomethacin were determined as log P value (octanol/water partition coefficient) and found to be 1.938±0.296 and 3.172±0.258 respectively. Preliminary solubility study was conducted for both the in various pure solvents. The solubility study indicated that both the drug chlorzoxazone and indomethacin were practically insoluble in water. Chlorzoxazone was sparingly soluble in methanol and phosphate buffer (pH 7.0), while soluble in chloroform, DMSO, DMF and freely soluble in sodium hydroxide solution and ammonia solution. Indomethacin was found soluble in methanol, ethanol, butanol, DMSO, DMF and freely soluble in sodium hydroxide solution.

To find out the influence of the pH on the solubility of chlorzoxazone and indomethacin, the pH dependent solubility studies were carried out at different pH ranging from 2.5-12.0 at 25±2°C and 35±2°C. The solubility of both the drugs were found to increase on increasing the pH of the solution. The solubility of chlorzoxazone was increase to 39.722±1.5 mg/ml at pH 12.08 i.e. 124.787 fold that of aqueous solubility at 25°C. The equilibrium solubility of indomethacin was found to 3.6399±0.3085 mg/ml at pH 7.96 i.e. 145.76 times of its aqueous solubility at 25°C. The pH of saturated solution of indomethacin was not increased above 7.96 because of buffering effect of indomethacin as it is a weak acid.

Hydrolysis profile of both the drugs were obtained at pH 3.0, 7.4 and 10.0. Semilogrithmic plots of concentration remaining versus time were linear indicating that the reaction was first order with respect to the drugs. The apparent first order hydrolysis rate constants determined from the slope of

such plots. Both the drugs were stable at lower to neutral pH, while degradation rate constant were found greater at pH 10.0 for chlorzoxazone and indomethacin.

The UV spectrum of chlorzoxazone and indomethacin was obtained by scanning 10 µg/ml solution in distilled water of respective drug between 200-400nm using Simadzu-1701 (Japan) UV spectrophotometer. The λ_{max} was found at 280 nm for chlorzoxazone and 265 nm and 319.5 nm for indomethacin. FTIR spectrum of both the drugs were obtained by potassium bromide disc method. The principle peaks of both the drugs were identified and matched with the standard FTIR of the respective drugs, confirming identity and purity of the drug.

Thermal behaviour of both drugs were determined by differential scanning calorimetry (DSC) curves. The endothermic peak was obtained at 194.399°C and energy for endothermic transition (ΔH) was 132.707 J/g for chlorzoxazone which was attributed to its melting. The endothermic peak for indomethacin was obtained at 161.533°C attributed to its melting point and energy for its endothermic transition was 78.426 J/g.

Crystal properties of both drugs were determined by performing X-ray powder diffraction (XRPD). Chlorzoxazone showed characteristic diffraction peaks at 8.781, 12.746, 19.814, 24.257, 25.700 and 27.461° at 20 scale. confirming that the drug was a high quality crystalline solid. Indomethacin showed characteristic diffraction peaks at 11.577, 16.969, 19.55, 21.806, 26.581 and 29.336° at 20 scale, attributed to γ form of indomethacin, indicating that the bulk powder was a high quality crystalline solid.

Percentage purity of both drugs was determined by performing assay by reported procedure and found to be 99.68% and 99.47% for chlorzoxazone and indomethacin respectively.

Standard calibration curves of chlorzoxazone and indomethacin was prepared in distilled water at their respective λ_{max} i.e. 280.0 and 319.5nm respectively.

The solubilities of chlorzoxazone and indomethacin were evaluated in five hydrotropes namely urea, resorcinol, nicotinamide, sodium benzoate and

sodium para-hydroxy benzoate in concentration of 0.2 M to 1.2 or 2.0M aqueous solution. The solubilities of both the drugs improved several folds in higher hydrotropes concentration. The solubility of chlorzoxazone in 1.2 M urea, resorcinol, nicotinamide, sodium benzoate and sodium pera hydroxy benzoate was 0.662 ± 0.045 , 4.18 ± 0.166 , 12.225 ± 0.529 , 17.215 ± 0.812 and 18.355±0.618 mg/ml at 25°C respectively, which was 2.080, 13.44, 38.405, 54.081 and 57.662 fold that of aqueous solubility of chlorzoxazone. The solubilizing power of different hydrotropes for chlorzoxazone could be ranked as sodium pera-hydroxy benzoate > sodium benzoate > Nicotinamide > Resorcinol > Urea.

Similarly solubility of indomethacin in 2.0 M urea, resorcinol, nicotinamide, sodium benzoate and sodium pera hydroxy benzoate was ...0.232±0.027, 0.750±0.103, 1.725±0.171, 2.000±0.227, 2.934±0.435 mg/ml respectively at 25°C, which was 9.291, 30.043, 69.065, 80.076, 117.499 fold that of aqueous solubility of indomethacin. The solubilizing power of different hydrotropes for indomethcin could be ranked as sodium para-hydroxy benzoate > sodium benzoate > Nicotinamide > Resorcinol > Urea.

The observation of phase solubility diagram of chlorzoxazone and indomethacin in aqueous solution of the five hydrotropes from concentration of 0.2 M to 1.2 (for chlorzoxazone) or 2.0 M (for indomethacin) revealed that the drugs were more soluble at higher hydrotrope concentration. The phase solubility diagram was Ap type. This shows that increase in total solubility of drug was not linear function of hydrotropic concentration. This positive deviation from linearity is due to formation of water-soluble complexes of higher order between drugs and solubilizer, typical of hydrotropism.

The stability constant $K_{1:1}$ and $K_{1:2}$ were calculated for drug hydrotrope complexation in 1:1 and 1:2 ratio by non-linear least square regression analysis of the solubility data.

The result indicate that increasing the hydrotrope quantity in system favors of high order complexes as the value of $K_{1:2}$ is greater than $K_{1:1}$. The lower values of K_{1:2} with increasing temperature indicate decrease in ligand hydrotrope ability. These findings suggest role of self-association in hydrotropy that decreases with increasing temperature.

The increasing hydrotrope concentration results in unassociated form of water to make cluster of the hydrotrope by hydrogen bonding and non-bonding interactions at the various centers of drug molecule. Thus charge delocalization along with an increase in π -cloud area on hydrotropic molecule would account partially for difference in apparent drug solubility in presence of various hydrotropes.

The plots of specific gravity versus hydrotrope concentration showed a negative deviation, that indicates an increase in partial molal volume upon aggregation, and this increase in volume may be due to expansion of the hydrocarbon portion of the molecule or its partial removal from the high compressive force of water. The positive deviation in the viscosity plots indicates that aggregate formation is associated with an increase in viscosity of hydrotrope concentration, which is in agreement with the self-association of phenolic compounds. The surface tension plots showed a moderate decrease in surface tension on increasing the hydrotrope concentration as hydrotropes are not surface active agents. The plots of refractive index versus hydrotrope concentration showed negative deviation. The deviation from linearity in specific conductance plots is strongly indicative of molecular aggregation. It was revealed from different studies that at lower hydrotrope concentration, weak ionic interactions while at higher hydrotrope concentration, the molecular aggregation seems to be the possible mechanism of hydrotropic solubilization.

UV spectral studies suggest that increase in solubility of chlorzoxazone or indomethacin in different hydrotrope solutions is not due to any complex formation between drug and hydrotrope molecules. Very slight shift in λ_{max} (±0.5 nm) might be due to minor possible effect of hydrotrope molecules on the electronic configuration of drug (chlorzoxazone/indomethacin) molecule.

Study of FTIR spectrum of drugs hydrotropes, their physical mixture and solubilized form suggested the possibility of hydrogen bonding between drug and hydrotrope.

A comparison among the endothermic transitions of drug and their solubilized product in various hydrotropes hydrotrope suggested that either complexation have occurred or crystalline nature of drug has been changed and/or it converted into amorphous form after drying in hydrotrope solution.

Remarkable difference between solubilized product and the physical mixture of drug and hydrotrope was found in X-ray powder diffractometry. The diffraction pattern of physical mixture was simply the sum of those of components. While in solubilized product characteristic peaks of drugs (chlorzoxazone or indomethacin) found to decrease intensity or disappeared, suggesting drug precipitate as amorphous powder in hydrotrope solution. These results confirm the findings of DSC.

Thermodynamic parameters for 1:1 and 1:2 complex formation were estimated from van't Hoff plots of the temperature dependence of stability constants. The value obtained by regression analysis provides information regarding the complex formation of drug hydrotropes systems.

The negative free energies of solubilization process are indicative of spontaneity of the process, more negative the free energy of complexation. the greater the solubility. The different value of ΔG corresponding to stability constants indicates different solubilization mechanism. The comparison of enthalpy and entropy changes to the free energy change of two type of complex formation also displayed considerable differences. The difference between free energy and enthalpy changes reflects the strength of, interaction or bonding strength of complexion. The value were found to be greater for 1:2 complex than 1:1 complex showing that the former is stronger than latter. The strength of these estimated ΔH value for both the drugs were not strong. hence it can be speculated that intermolecular forces other than hydrogen bonding are involved in solubility enhancement of chlorzoxazone and indomethacin.

The solubilization of drugs cannot be attributed to complexation. The hydrotrope self-association significantly plays a role in solubilization mechanism. Additionally, high concentration of hydrotropic in conjugation with self-association change the solvent nature of water and hydrotropic system behaves as a co-solvent system in which one component is solid in solution rather than liquid.

The effect of HP β-cyclodextrin on aqueous solubility of chlorzoxazone and indomethacin was evaluated using the phase solubility method. The phase diagram was classified as type A_L, which denotes a linear increase in solubility.

The solubility enhancement attained by 20% W/V HP β-CD was 14.584 and 15.184 mg/ml at 25°C and 37°C respectively that was 45.8 fold and 43.724 fold, of aqueous solubility of Chlorzoxazone at the respective temperature. Based a phase solubility diagram, the association constants for the inclusion complex was determined assuming 1:1 ratio of complex formation.

Slopes and intercepts were determined by performing linear regression analysis of phase solubility data. The regression equations for calculation of $K_{1:1}$ at various temperature were found to be Y (25°) = 0.5507x + 10.484 and Y (37°) = 0.5681x + 11.428. Association constants $K_{1:1}$ were found to be $652.91~\text{M}^{-1}$ and $642.26~\text{M}^{-1}$ at 25~°C and 37~°C respectively. The complex stability decreases with increase of temperature. The complexation efficiency of HPβ-CD was found to be 1.226 and 1.315 at 25°C and 37°C respectively.

Van't Hoff plot was constructed to estimate thermodynamic parameters of chlorzoxazone HP β -CD complex. The values of entropy change ΔS (disordering or bond breaking) were negative, revealing the possibility of an increased ordering of species by complexation. Both values of the free energy change ΔG and the enthalpy change ΔH (bonding strength) were negative, indicating the spontaneity and exothermic nature of chlorzoxazone-HPβ-CD complexation solubilization. Large negative AH suggests that chlorzoxazone hydroxypropyl β-cyclodextrin inclusion is an enthalpy driven process.

The infrared spectrum of chlorzoxazone hydroxy propyl β cyclodextrin complex was similar to the pure hydroxypropyl β-cyclodextrin but dissimilar to chlorzoxazone and a physical mixture of chlorzoxazone and hydroxypropyl βcyclodextrin suggesting complex formation.

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X-ray diffraction patterns of chlorzoxazone, hydroxypropyl-βcyclodextrin and its complex showed that they were amorphous. The hydroxypropyl-β-cyclodextrin complex was dissimilar to physical mixture, thus suggesting complex formation A comparison among the endothemal transitions suggesting complex formation. The complex showed no evidence of sharp chlorzoxazone response at 194.399°C.

Similar to chlorzoxazone, increasing the amounts of hydroxypropyl β -cyclodextrin increased the amount of indomethacin going into water, improving aqueous solubility of indomethacin. The phase solubility diagram was found to be A_L type suggesting 1:1 stoichiometry of the inclusion complex. The solubility enhancement attained by 20% W/V HP β -CD was 2.095 and 2.323 mg/ml at 25°C and 37°C respectively that was 83.884 fold and 81.819 fold of aqueous solubility of indomethacin at the respective temperature.

Slopes and intercepts were determined by performing linear regression analysis of phase solubility data. From figure the regression equations for calculation of $K_{1:1}$ at various temperature were found to be Y (25°) = 0.0412x -0.0663 and Y (37°) = 0.0457x - 0.0067. Association constants $K_{1:1}$ were found to be 615.6603 M^{-1} and 603.4638 M^{-1} at 25°C and 37°C respectively. For 1:1 indomethacin-HP β -CD complex. The complexation efficiency of HP β -CD was found to be 0.043 and 0.048 at 25°C and 37°C respectively.

Van't Hoff plot was constructed to estimate thermodynamic parameters of indomethacin HP β -CD complex. Large negative ΔH suggests that indomethacin-hydroxypropyl β-cyclodextrin inclusion is an enthalpy driven process. The negative value obtained for both ΔH and ΔS would seem to suggest that stabilizing interactions in the complex were hydrophobic as well as through hydrogen bonding.

The infrared spectrum of the hydroxypropyl β-cyclodextrin complex was similar to the pure hydroxypropyl β-cyclodextrin but aissimilar to indomethacin and physical mixture of indomethacin and hydroxypropyl β-cyclodextrin suggesting complex formation.

Analysis of X-ray diffraction patterns of indomethacin, hydroxypropyl β -cyclodextrin, physical mixture and their complex showed the indomethacin was a γ-form crystalline powder evident from characteristic crystalline reflection. hydroxypropyl β -cyclodextrin and its indomethacin complex were amorphous. The hydroxypropyl β -cyclodextrin complex was dissimilar to physical mixture suggesting complex formation.

A study of thermal transitions of indomethacin, hydroxypropyl β-cyclodextrin, physical mixture and their complex revealed complex formation as evident from shift in peak temperature in the complex thermogram and had no evidence of a strong indomethacin response at 161°C from γ-from of indomethacin, thus suggesting complex formation.

Finally it is concluded that the solubility enhancement of chlorzoxazone /indomethacin was attributed to inclusion type complex formation of 1:1 stoichiometry evident by the A_L type of Phase Solubility Diagram. The stoichiometry of chlorzoxazone / hydroxypropyl β-cyclodextrin or indomethacin / hydroxypropyl β-cyclodextrin complexes cannot be derived exclusively from simple phase solubility studies. Self-association of surface-active drugs, Lipophylic drug molecules and drug/cyclodextrin complexes as well as solubilization through non-inclusion interactions with drug/cyclodextrin complexes, will influence both the shape and mathematical interpretation of the phase solubility diagrams.

In the study five cosolvent namely PEG-400, PEG-200 ethanol, propylene glycol and glycerin were used to solubilize indomethacin and chlorzoxazone. They resulted in various degree of improvement of solubilization of both drugs. An increase in concentration of cosolvent produces an exponential increase in drug solubility. Thus, solubilization effect was much significant at high cosolvent concentrations.

The solubilizing power of the cosolvents were calculated by the slope of zero intercept curve of semi-log plot, plotted between log of solubility enhancement ratio verses fraction of cosolvent. This facilitates comparison among both drug as well as different cosolvent. The solubilizing power of the five cosolvents PEG-400, PEG- 200, Ethanol, Propylene glycol and glycerin was found 2.9125, 2.6381, 2.6991, 1.9671, 0.8255 for chlorzoxazone and 3.5731, 3.1526, 2.7589, 2.086, 1.8092 for indomethacin at 25° C. Thus the five cosolvents can be arranged regarding their solubilization power for chlorzoxazone according to the following rank: PEG-400 > Ethanol > PEG-200 > Propylene glycol> glycerin and for indomethacin according to the following rank: PEG-400 > PEG-200 > Ethanol > Propylene glycol> glycerin. This order is an agreement with the ranking of cosolvent with respects to their reported dielectric constant or solubility parameter.

Dielectric constants of the solvents show that the polarity of the solvents varies as water > glycerin > propylene glycol > ethanol > polyethylene glycol 200 > polyethylene glycol 400. Solubility of the drugs decreases with an increase in the polarity of solvents. Thus, polarity of the solvent is an important factor governing the solubility of the drugs. Hydrophobicity of the solvents, measured as octanol-water partition coefficients (log P), also showed that the solubility increases with the hydrophobicity of the solvent. However, polarity and hydrophobicity are not the only factors involved, the ability of the solvent to form hydrogen bonds with the hetero-atoms in the drug molecule is another important factor governing the solubility of drugs, the same is true for higher solubility in ethanol. The greater solubility of drugs in ethanol than in propylene glycol suggests that the solubility is also governed by the intermolecular interactions between the solvent molecules, which are expected to be stronger in glycols than in alcohols. In the case of glycols, the increase in solubility in moving from propylene glycol to polyethylene glycol suggests that the hydrophobic interactions are more important in governing the solubility of the studied drugs in glycols. The exceptionally high solubility of drugs in polyethylene glycol 400 is probably because of extensive hydrophobic interactions with the solvent because polyethylene glycol 400 has a long nonpolar part compared with other solvents 6.任所转为5年30日至,韓蘇劉伊德報子起的人民政策和6.7

To investigate the effect of temperature on the solubility of chlorzoxazone and indomethacin by these cosolvents, the solubility study was

performed at two temperature levels i.e. 25°C and 37°C. Elevation of the temperature was accompanied by a minor but detectable increase in the solubility of chlorzoxazone and indomethacin.

The free energy change (ΔG) associated with the solubility of chlorzoxazone was found to be -16.02, -15.02, -13.44, -11.51 and -4.56 For PEG-400, PEG-200, ethanol, propylene glycol and glycerin respectively at 25°C. The free energy change (ΔG) associated with the solubility of indomethacin was found to be -20.92, -19.85, -16.41, -14.01 and -8.82. For PEG-400, PEG-200, ethanol, propylene glycol and glycerin respectively at 25°C indicating spontaniety of process.

The breaking up of water clusters surrounding the non-polar molecule requires heat ($+\Delta H$). Moreover, the dissolution process is endothermic one when ΔH is positive. Therefore, an increase in temperature from 25° to 37°C caused an increase in chlorzoxazone or indomethacin solubility.

Solubilization behavior of chlorzoxazone and indomethacin in various proportions of mixed cosolvent systems Viz. Ethanol/PEG-200, Ethanol/PEG-400, Ethanol/propylene glycol and PEG-200/PEG-400 was also determined. Again, an exponential increase is solubility of both the drugs was observed when the proportion of high solubilizing power cosolvent is increased. Maximum drug solubility was observed in PEG400-ethanol mixtures in the case of both drugs.

The solubilization of chlorzoxazone and Indomethacin was studied in micellar solutions of three surfactants namely Tween-80, Tween-20 and Gelucire 44/14. Based on reported data on safe use in injectable formulation, Tween-80 was used up to the concentration of 5.0% v/v and Tween-20 was used up to 0.5% v/v. Gelucire 44/14 a well-defined mixture of mono-, di- and tri-glycerides and mono- and di-fatty acid esters of polyethylene glycol was used up to 20.0% w/v to find out its possible use in solubility enhancement of chlorzoxazone and Indomethacin. The results showed that, irrespective of the surfactant type, the solubility of chlorzoxazone and Indomethacin increased linearly with increasing surfactant concentration, as a consequence of the association between the drug and the micelles.

The values of molar solubilization capacity (χ) were obtained from the slope of solubility curves of drug(s) with the three surfactants (Stot vs. Csurf). The highest value of molar solubilization capacity (χ) was obtained with Gelucire 44/14, χ = 0.201, followed by tween-20, χ = 0.183, and finally tween-80, χ = 0.145 at 25°C. Thus effectiveness of the three surfactant could be ranked as solubiliser for chlorzoxazone as Gelucire 44/14 >Tween-20 > Tween-80.

For indomethacin, the values of molar solubilization capacity (χ) was highest value with tween-20, χ = 0.941, intermediate with tween-80, χ = 0.093, and lowest with Gelucire 44/14, χ = 0.065 at 25°C. Thus effectiveness of the three surfactant could be ranked as solubilizer for Indomethacin as Tween-20>Tween-80>Gelucire 44/14.

The increased solubility of the drug in non-ionic micellar solutions is not only a consequence of micelle-drug interaction, but also of the fraction of surfactant in the micellar form. For the nonionic surfactants, the molar fraction of surfactant in the micellar form is higher, since the CMC is much lower. In order to make this statement clear, the molar solubilization capacities of the surfactants, as well as the partition coefficients, were calculated. The molar micelle-water partition coefficients, K_M tween-20 = 97.419 and K_M tween-80 =77.221. K_M Gelucire-44/14 = 2884.4 for chlorzoxazone. Accordingly, the tendency of chlorzoxazone to partition preferentially with the Gelucire-44/14 micelle is higher than the tendency to partition with the tween-20 or tween-80 micelle. The tween-80 presented the lowest values of χ and $K_{M\cdot}$ as discussed earlier. For indometacin the molar micelle-water partition coefficients, KM tween-20 = 13448 K_M tween-80 =1326 and K_M Gelucire-44/14 = 937.1, the tendency of indomethacin to partition preferentially with the tween-20 micelle is higher than the tendency to partition with the tween-80 or Gelucire-44/14 micelle. The Gelucire-44/14 presented the lowest values of χ and $K_{\rm M}$.

It was observed that these surfactants were found to be very less effective solubilizer for solubilization of chlorzoxazone but fairly good solubilzer for indomethacin. This may be due to self-association of

indomethacin molecules and co micellization with surfactants. The process of transfer of drug from water to surfactant solution cannot be envisaged entirely as a simple solution mechanism, but rather as an interaction between surfactant and cosolute i.e. comicellisation. Indomethacin was also known to form micelle like aggregates or mixed micelles. From the thermodynamic point of view, the solubilization can be considered as a normal partitioning of the drug between two phases, micelle and aqueous. ΔG was negative, indicating spontaneous solubilization.

The negative value of ΔG can be arranged in following rank Gelucire 44/14 > Tween-20 > Tween-80 for chlorzoxazone and Tween-20> Tween-80>Gelucire 44/14 for indomethacin. Positive ΔH values show the dissolution process is endothermic. The values of ΔS were also positive indicating greater the randomness and disordering of the system or the solute molecules become more randomly spread through the dissolution medium during solubilization process.

Based on the results of solubility determination studies, three formulation of aqueous injection of chlorzoxazone namely C-1, C-2 and C-3 were prepared. These parenteral formulations may be useful wherein the oral administration of chlorzoxazone is contraindicated or to uncooperative patients. At the same time there will be possibility of reducing the drug dose as well as the adverse effect.

Though it was difficult to attain desired dose 500 mg in 3 ml of injection volume, so higher concentration of PEG-400 with 10% ethanol was taken as cosolvent mix and 500 mg of chlorzoxazone was achieved in 5 ml of injection volume. The combination of PEG-400 and ethanol are common cosolvents vehicle that are considered safe for use in preparation of parenteral solutions. 5% sodium benzoate was added as hydrotropic agent, which also served as buffer. In C-2 formulation benzoate buffer was replaced with 0.02 M phosphate buffer (pH 7.8). In formulation C-3 a ratio of 80% PEG-400 and 4% benzyl alcohol could be able to dissolve a high concentration of chlorzoxazone that is 400 mg / 3 ml. Benzyl alcohol 4% was added as it has synergistic effect in solubilization along with benzoic acid/sodium benzoate

and also reduce pain on injection due to its local anesthetic properties. In each formulation 0.1% sodium metabisulfite was used as antioxidant.

On the basis of solubilization studies seven formulation of indomethacin with target strength of 50 mg in 2 ml or 3 ml injection volume were developed utilizing cosolvent and hydrotrope both and/or surfactant. These parenteral formulations may be useful in patient with rheumatic disorder, peptic ulcer etc. wherein the oral administration of indomethacin in contraindicated, with the possibility of reducing the drug dose as well as the adverse effect.

Cosolvents PEG-400, PEG-200 and PG were selected on the basis of their solubilizing power and in their acceptable concentration/proportion for injectable formulation. Among hydrotropes only sodium benzoate was selected, though the drug showed highest solubility in sodium p-hydroxy benzoate, but it was not used because presently there is no injectable formulation utilizing this hydrotrope so its acceptability was not known. Nicotinamide was also showed promising solubility enhancement, but it was also not used because of its high cost. Benzyl alcohol (4%) was added to each formulation as it has synergistic solubilization effect and also reduces pain on injection due to its local anesthetic properties. In formulation I-4 instead of benzyl alcohol, tween-80 (5% w/v) was utilized. Other surfactants such as tween-20, was not used because it produces yellowish colour during solubilization studies and gelucire 44/14 was not used because alone it was not able to produce desirable drug concentration and in combination of cosolvent, it was insoluble and produces opalescence.

Additionally three preparation of indomethacin with 1mg/ml target strength were also developed utilizing hydrotrope alone. In each formulation, 0.1% sodium methabisulfite was added as antioxidant.

All the developed formulations were assayed for drug content and found within $\pm 1\%$ limit of stated drug amount. The pH of formulations were also found to be within limit. When observed against black and white background under diffused fluorescent light with naked eye all the sealed vials were found to be free from particulate matter.

Result of sterility testing, performed on the three vials of each formulation by direct inoculation method, showed that they were sterile as there was no turbidity or growth was observed in each culture tube after 7 days of inoculation. Thus it was concluded that the formulations developed were satisfactory and proceeded for further studies.

The physical stability studies of chlorzoxazone formulations showed that except slight colour change in formulation C-2, all the formulations remained unchanged with respect to colour stability. No turbidity or precipitate formation was observed in formulation C-3 at all storage conditions. But formulation C-1 and C-2 showed slight precipitation on 31st day and 39th day respectively when stored in temperature cycling with shaking (TCS) condition, while at room temperature and freezing temperature (4±2°C) in dark there was no precipitate formation upto 45 days. The pH of all the formulations remained within the specified limit upto 45 days.

The physical stability studies of indomethacin formulations showed that except slight colour change in formulation I-1b, I-4 and IPHB, all the formulations remained unchanged with respect to colour stability. No turbidity or precipitate formation was observed in formulation I-2b, I-3a, I-3b, I-4 at all storage conditions. But formulation I-1a, I-1b, I-2a, ISB, IPHB and INMD showed slight precipitation on 13th, 19th, 43rd, 41st, 14th, and 42nd day respectively when stored in temperature cycling with shaking (TCS) condition and I-1a, I-1b and IPHB showed precipitation on 31st, 39th and 31st day respectively when stored at freezing temperature in dark. At room temperature in dark there was no precipitate formation upto 45 days in all the ten formulations. The pH of all the formulations remained within the specified limit upto 45 days.

The selected formulations were stored at 37±2°C, 45±2°C and 60±2°C and the residual drug content of formulation was measured after 1, 3, 7, 14, 21, 30 and 45 days. The plot of log % residual drug vs. time (days) was found in the straight line, which indicates the degradation, follows first order kinetics.

The rates of degradation of formulation were faster at elevated temperatures. The degradation rate constant was minimum for C-3

formulation i.e. 8.33, intermediate for C-2 i.e. 9.15 and maximum for C-1 i.e. 9.76 days⁻¹x10⁴ at 37±2°C.

The shelf life of formulations were found to be 175.083, 163.677 and 211.273 days for formulation C-1, C-2 and C-3 respectively at 25±2°C. Thus among the three formulations C-3 formulation was found to be most stable.

Among the formulation of indomethacin the degradation rate constant was found to be lowest for the formulation I-2b. The shelf life of formulations were found to be 171.0, 172.2, 311.6, 318.8, 247.4, 255.7, 218.6, 185.1, 109.8 and 227.7 days for formulation I-1a, I-1b, I-2a, I-2b, I-3a, I-3b, I-4, ISB, IPHB and INMD respectively at 25±2°C. Thus among all the ten formulations I-2b formulations was found to be most stable. Among cosolvent formulations, formulations containing higher concentration of cosolvent were found to be more stable i.e. I-1b, I-2b, I-3b. Among formulation containing hydrotropes. the formulation INMD (with nicotinamide) was found to be most stable.

On the basis of the result of physical and chemical stability testing, the three promising formulations of chlorzoxazone namely C-1, C-2 and C-3 and four promising formulation of indomethacin namely I-1b, I-2b, I-3b and I-4 were selected for in vitro evaluation.

The selected chlorzoxazone formulations when diluted with normal saline or 5% dextrose solution showed immediate precipitation in all the formulations, however, in higher dilution ratio, the precipitate was redissolved. In these formulations, high concentration of chlorzoxazone was solubilized by incorporation of higher cosolvent concentration (PEG-400 upto 80% + ethanol 10%), thus after dilution, the solubility of chlorzoxazone reduce exponentially while the volume increase linearly. Hence, resulted in precipitation of chlorzoxazone.

Dilution of a formulation of indomethacin with normal saline or 5% dextrose solution did not result in immediate precipitation of transient cloudiness under all condition of dilution. The observation reveal that in formulation I-3b all the test remained clear for at least 1 h, while the other formulation remained clear for about 0.5 h, with normal saline as well as 5% dextrose solution, but slight to clearly visible microcrystalline precipitate was appeared afterwards at lower dilution ratio.

The haemolytic activity of indomethacin and chlorzoxazone in different formulations at different drug concentrations was studied. The data clearly shows that all the selected formulations exhibit a haemolytic effect. The formulation of both the drugs with 500 µg/ml drug concentration resulted in little, nearly 11% haemolysis. Among chlorzoxazone formulations haemolytic behaviour can be ranked as C-3>C-2>C-1 and among the formulations of indomethacin, the haemolytic behaviour can be ranked as I-2b>I-4>I-1b>I-3b.

All the hydrotropes studied, exerted a negligible haemolytic effect at concentration below 0.4 M but highly significant haemolysis above 0.4 M of the hydrotrope concentration. All the hydrotrope produces nearly 80% haemolysis when studied in water in the concentration of 2 M. The haemolytic activity of these hydrotropes can be ranked as Sodium p-hydroxy benzoate > nicotinamide > sodium benzoate. Among cosolvents PG, PEG-200 and PEG-400 showed about 100% haemolysis when used in concentration above 60%, while ethanol showed about 100% haemolysis when used in concentration above 10%. Thus the haemolytic activity of these cosolvents can be ranked as ethanol>PEG-400>PEG-200>PG.

In a parallel study, the effect of varying concentration of sodium chloride (0.45-1.8%) on haemolytic behaviour of the hydrotrope in concentration range 0.2-2 M and upto 70% of cosolvents was also studied. Sodium chloride at the 1.8% concentration significantly reduced the haemolytic activity of sodium benzoate, nicotinamide, sodium p-hydroxy benzoate and ethanol, while in case of PG, PEG-200 and PEG-400 0.9% NaCl reduces the haemolytic activity significantly.

The bioavailability was assessed and compared using drug plasma profile plot between drug plasma concentration verses time. The time of maximum plasma concentration of Clz was about 1.5 hours and peak plasma concentration was 34.56±5.21 µg/ml. The time of maximum plasma concentration (T_{max}) for all injectable formulation C-1, C-2 and C-3 were found

to be 0.25 hr. and peak plasma concentration (C_{max}) were found 44.56±6.82, 43.38±6.45 and 42.86±6.17 respectively.

The area under the curve (AUC₀₋₁₂) was obtained by the integration of curve from 0.25 to 12 hrs and found to be 151.49±7.26, 141.58±6.53, 146.55±6.34 and 126.60±5.99 for C-1, C-2, C-3 and Clz oral formulation respectively. Thus, in comparison to Clz oral formulation all the three injectable formulation namely C-1, C-2 and C-3 have 117.8%, 110.1%, and 114.0% bioavailability respectively. It can be concluded that the developed parenteral formulations of chlorzoxazone have better bioavailability with less variation in pharmacokinetic parameters than the oral dosage form.

The profiles for all injectable formulations of indomethacin were comparable but distinct from Idicin capsule. Idicin lacked a smooth profile. which was attributed to prolonged drug absorption due to entrohepatic recirculation of indomethacin.

The area under the curve (AUC₀₋₁₂) was found to be 55.88 ± 2.53 , 54.92±2.67, 53.68±2.34, 56.75±2.81 and 48.26±2.81 for I-1b, I-2b, I-3b, I-4 and Idicin formulation respectively. Thus, in comparison to marketed capsule formulation Idicin all the four injectable formulation namely I-1b, I-2b, I-3b and I-4 have 115.8%, 113.8%, 111.2% and 117.6% bioavailability respectively.

All the four injectable formulation have immediate peak plasma concentration T_{max} =0.25 hrs in comparison to Idicin which have $T_{\text{max}} = 4.0 \text{ hrs.}$

To study the significance level for the variation among different formulations, students t test was applied. It clearly indicates that the formulations given orally shows significant variation when compared with any of the injection formulations. This suggests that the difference in bioavailability is small but significant. So, it can be concluded that the developed parenteral formulations have better bioavailability with less variation in pharmacokinetic parameters than the oral dosage form.

Finally it is concluded that cosolvents were most powerful tool for solubilization of water insoluble drugs like indomethacin and chlorzoxazone. The solubilizers can be ranked according to their solubilizing effect for chlorzoxazone and indomethacin in their parenterally accepted concentration as cosolvents > hydrotropes > complexants > surfactants.

All developed formulations of chlorzoxazone and indomethacin have better bioavailability with less variation in pharmacokinetic parameters than oral dosage form. Additional formulations of indomethacin (1 mg/ml), developed using hydrotrope alone showed good results regarding their physical and chemical stability and in vitro dilution and haemolysis studies. These formulations may further proceed for clinical studies as these formulations are much needed for closing hemodynamically significant patent ductus areteriosus (PDA) in premature infants.

LIST OF PUBLICATION

- 1. A.K. Jain (2007). Solubilization of indomethacin using hydrotropes for aqueous injection. European Journal of Pharmaceutics and Biopharmaceutics. (Accepted).
- A.K. Jain and S.K. Jain (2007). Poster presentation on "Solubility enhancement of chlorzoxazone using various solvent systems. National Seminar on Controlled and Targeted Drug Delivery Nanotechnology, 17th and 18th March, Organized by DOPS, Dr. H.S. Gour University, Sagar (M.P.).
- A.K. Jain, S.K. Jain and S. Prajapati (2007). Enhancement of solubility of chlorzoxazone and indomethacin and physicochemical characterization of their molecular inclusion with hydroxypropyl-β-cyclodextrin complexes. Current Pharma Research Journal. (Communicated).
- 4. A.K. Jain, S.K. Jain and S. Prajapati (2007). Solubilization of chlorzoxazone using cosolvents. *Indian Journal of Pharmaceutical Education And Research*. (Communicated)